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Further Development of Chemical and Biological Processes for Production of Bioethanol: Optimisation of Pre-treatment Processes and Characterisation of Products

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Abstract

The efficiency of several processes for pre-treatment of lignocellulose has been investigated to provide suitable feedstock for enzymatic hydrolysis and fermentation. Wet oxidation (with and without alkaline) has been investigated for wheat straw, birchwood, and willow treating 60 g/L. The conditions for willow and birchwood was selected based on the optimal conditions for wheat straw. Three different harvest years of wheat straw were included to evaluate the effect of crop variation from year to year. Comparative studies were made using steaming and steam explosion of wheat straw.

Alkaline wet oxidation fractionated wheat straw efficiently into solubilised hemicellulose and a highly convertible cellulose fraction. High oxygen (12 bar) during treatment and low lignin in treated fibres resulted in highly convertible cellulose. Different optimal reaction conditions were found for different harvest years. For straw 1993 and 1997, the conditions were 185°C, 15 minutes resulting in 9-10 g/L solubilised hemicellulose and 63-67% cellulose convertibility. For straw 1994, the conditions were 195°C, 5 minutes resulting in 7.5 g/L solubilised hemicellulose and 96% cellulose convertibility. For willow, the optimal pre-treatment was wet oxidation without alkaline using 185°C, 15 minutes (from 60 g willow/L). These conditions gave 8.2 g/L hemicellulose in solution and 50% cellulose convertibility, which was lower than that of wheat straw. High recoveries were obtained for willow compared to wheat straw. Addition of alkaline significantly decreased fractionation and degree of convertible cellulose. For birchwood, the best process conditions were hydrothermal treatment (without oxygen and alkaline). At 200°C and 15 minutes, 8 g/L hemicellulose was solubilised with high recoveries for both polysaccharides, however, poor cellulose convertibility was found (<30%). The addition of oxygen (wet oxidation) also resulted in a high fractionation but decreased the recoveries. Alkaline wet oxidation resulted in the highest cellulose convertibility but low content of solubilised hemicellulose (< 4 g/L).

In general, formation of furfurals was avoided by adding alkaline to wet oxidation. In the absence of alkaline, furfural formation was higher (up to 130 mg/100 g wheat straw) than that of steam explosion (43 mg/100 g straw). Formation of carboxylic acids was highest during alkaline wet oxidation and highest for birchwood (up to 8 g/L). Minor amounts of phenolic compounds were identified after alkaline wet oxidation of wheat straw. However, some residual total organic carbon presumably high molecular weight components remained unidentified.

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Contents

Preface

This report presents the significant results of the work performed in the subproject "Optimisation of Pre-treatment Processes and Characterisation of Products" carried out in the Plant Biology and Biogeochemistry Department at Risø National Laboratory from 01/05/96 to 31/12/98. This subproject was part of the project "Further Development of Chemical and Biological Processes for Production of Bioethanol" (J.No. EFP 1383/96-0007) carried out in collaboration with The Technical University of Denmark (DTU), Lyngby, funded by the Danish Ministry of Energy and Environment. The work on straw from 1997 was partly carried out within the project: Biobased Packaging for Foods (funded by non-food program, Danish Directorate for Development, Ministry of Food, Agriculture and Fisheries).

The goal of project was to further evaluate some biological and chemical processes for bioethanol production from fractionated lignocellulose, i.e. wheat straw, birchwood, willow, and sugar beet pulp. Several different pre-treatment processes, i.e. wet oxidation (with or without alkaline), hydrothermal treatment (with or without alkaline), steaming, and steam explosion, were studied. The aim was to obtain 1) a solid cellulose-rich fraction accessible for enzyme treatment to hydrolysis to glucose and 2) a liquid hemicellulose-rich fraction without producing microbial inhibitors and at the same time a high recovery of the polysaccharides. The pre-treated solutions and suspensions were delivered to DTU for fermentation to ethanol. Analytical tools were developed throughout the project in order to characterise the carbohydrate fractions. Published works originating from this project have been enclosed as appendices.

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The authors would like to thank Dr. Jürgen Puls, Institute of Wood Chemistry, Hamburg, Germany for analysis of some acid hydrolysates for monosaccharide composition using borate-complex anion-exchange HPLC and for performing the steaming experiments. The assistance from Tomas Fernqvist, Birgit Jensen, Helene B. Klinke, Ingelis Larsen, Jette Bruun Nielsen and Anders Woidemann at Risø National Laboratory is also gratefully acknowledged.

1 Introduction

A new concept for ethanol production based on the combined utilisation of hemicellulose and cellulose from wheat straw has been developed (Ahring *et al*., 1997). The concept was based on the combination of wet-oxidation pre-treatment of wheat straw where the hemicellulose fraction was fermented to ethanol by a new extreme thermophilic anaerobic bacterium *Thermoanaerobacter mathranii* strain A3M1 and the cellulose fraction by mesophilic yeast after enzymatic treatment. The effluent (solid and liquid fraction) from the wet oxidation was hydrolysed enzymatically by commercial cellulases (Ahring *et al*., 1999) after which the hydrolysed cellulose (glucose) was converted to ethanol by *Saccharomyces cerevisiae* (**Figure 1**). The hemicellulose (mainly xylose and xylan) was fermented to ethanol by *T. mathranii* A3M1 at 70°C with a high productivity (Larsen and Ahring, 1998; Ahring *et al*., 1999). The wastewater streams could be converted to biogas in an Upflow Anaerobic Sludge Blanket (UASB) reactor for energy (data not published). The purified wastewater from the UASB reactor is recirculated and reused as process water for the wet oxidation process (data not shown). The pre-treatment, hydrolysis and fermentation step of the hemicellulose fraction for production of ethanol have been tested separately, whereas mesophilic yeast fermentation of the glucose fraction, distillation of ethanol and wastewater treatment is still in the experimental stage.

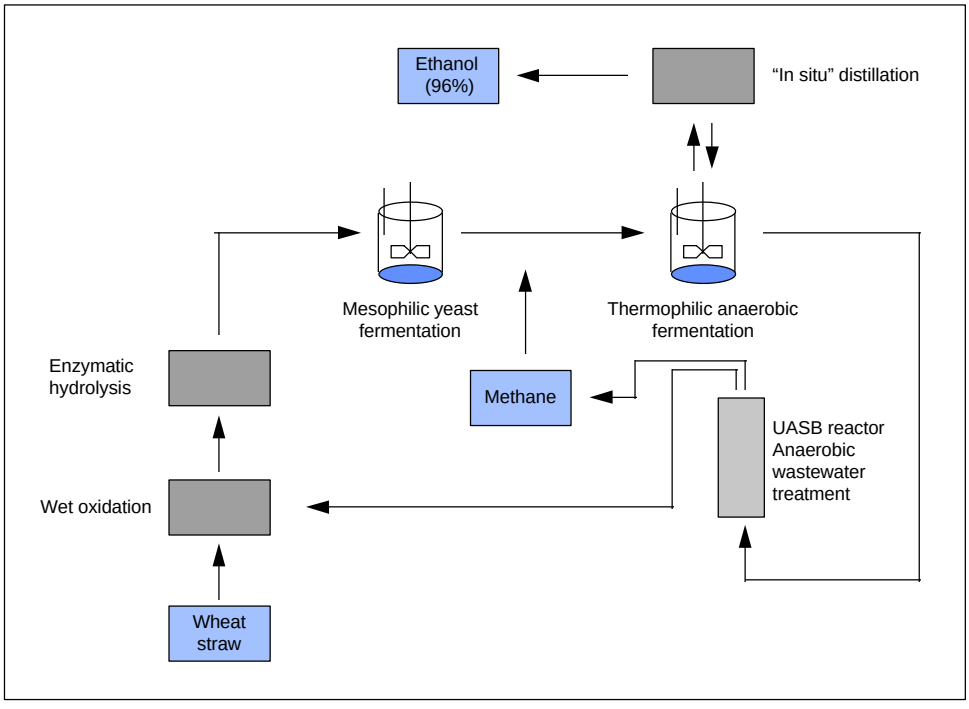

Figure 1. *The schematic flowsheet of the overall novel process concept.*

The wet oxidation pre-treatment has been shown to an effective fractionation method of wheat straw into bio-convertible polysaccharides of cellulose and hemicellulose (Bjerre *et al.*, 1996a; Schmidt and Thomsen, 1998). The principle of the treatment is illustrated in **Figure 2**. Apart from polysaccharides available for fermentation some degradation products were formed during the treatment. These were identified as phenolic compounds and low molecular weight carboxylic acids that might be potential inhibitors during fermentation of the sugar fractions (Klinke *et al*., 1999).

The inhibitors were produces by decomposition of the plant constituents (lignin, hemicellulose, cellulose, wax, and pectins) due to thermal degradation and oxidation. By alkaline wet oxidation, the production of furans seemed to be avoided (Bjerre *et al.*, 1996a; Schmidt and Thomsen, 1999) due to further oxidation of these compounds to carboxylic acids (**Figure 2**) (Klinke *et al*., 1999). The furans, 2-furfural and 5-hydroxymethyl-2-furfural are common degradation products from the more investigated pre-treatment method steam explosion (Buchert *et al*., 1988; von Sivers *et al*., 1994) due to thermal decomposition of xylose and glucose, respectively (also elucidated in **Figure 2**).

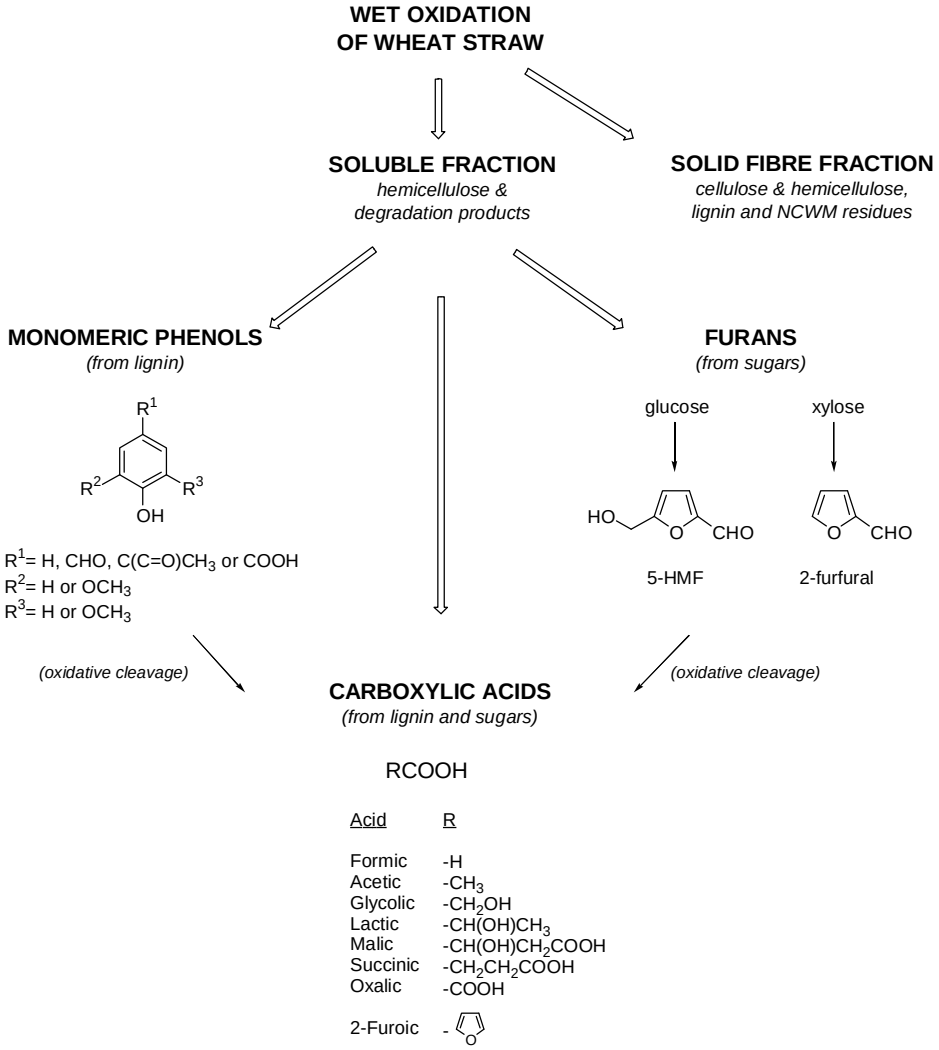

Figure 2. *Principle of wet oxidation when treating wheat straw. NCWM = non-cell wall material; 5-HMF = 5-hydroxymethyl-2-furfural. (Klinke et al., 1999).*

Steaming or steam explosion is an extensively investigated pre-treatment method. A vast amount of literature can be found, treating several types of raw materials (Clark and Mackie, 1987; Eklund *et al*., 1995; Heitz *et al*., 1991; Schultz *et al*., 1983). An advantage of steam pre-treatment is that it is one of a very few fractionation methods that have been tested in pilot scale. Commercial equipment, such as the batch digester of the Masonite gun type and continuous reactors such as the Stake Technology and the Sunds AB reactors are available. In Finland, a xylitol production plant has operated since 1988 using hemicellulose liquor derived from steam treatment of hardwoods (Schultz *et al*., 1983). Steam pre-treated material is well suited for enzymatic hydrolysis when hardwoods, agricultural residues or

herbaceous crops are used. The yields of hydrolysing cellulose into glucose are often high, around 90%. Typical temperatures range from 180 to 250°C and residence times from 10 s to 1 h. A high temperature is usually accompanied by a short residence time and *vice versa*. The extraction of hemicelluloses increases with increasing temperature from 170-210°C. However, when the temperature approaches 200°C, a drop of the amount of xylose and xylo-oligomers in the liquid fraction can be observed, due to degradation. Impregnation with sulfuric acid or sulfur dioxide *prior* to steam pre-treatment enhances the survival of pentose sugars and, hence, improves the sugar recovery, when compared with material subjected to the same process conditions, and no catalyst. Up to about 65-80% of the pentose sugars can be found in the water-soluble fraction after a steam pre-treatment for hardwood (Eklund *et al.*, 1988; Greeg and Saddler, 1996). When softwoods are considered, steam pre-treatment is not very efficient in the absence of catalyst. However, by adding the catalysts H_2SO_4 or SO_2 also enhanced the hemicellulose and the cellulose recoveries for softwoods. Yields of around 65%, regarded as monomeric sugars, have been reported (Greeg and Saddler, 1996).

The nature of a cereal crop like wheat varies considerably from year to year. This is very dependent of the weather conditions, such as sun, precipitation, temperature and wind. These circumstances also have a considerably effect on the yield of wheat grain on the field. Furthermore, the type and nature of the soil and use of fertilisers also influence the quality of the wheat grain and straw.

In this and the previous and project (J. No. EFP-96 1383/0007 and EFP-94 1383/0004) wheat straw from four different harvest years (1990, 1993, 1994 and 1997) had been applied. The straws from the 3 first years were procured very early in the previous project (J. No. EFP-94 1383/0004), when it could not be foreseen that there was a need for large amount of substrate for fermentation purposes at DTU. In order to avoid the need for optimisation of wet oxidation of a new wheat straw all the time, 50 kg of wheat straw 1997 was acquired. This should be enough raw material for several years of research, hence, the wet oxidised wheat straw delivered to DTU should be of a more uniform character.

In this study, wet oxidation pre-treatment was investigated as an alternative to steaming and steam explosion. Wet oxidation of wheat straw from different harvest year was optimised and compared individually. Wet oxidation was also performed on other lignocellulosic materials, birchwood and willow. Birchwood, willow, and wheat straw used in this project were not only selected from reasons of availability, but also due to the fact that they represent lignocellulosic material of high (birchwood) and low (wheat straw) acetyl content which may have a great influence on the pre-treatment method to be used. Hydrothermal treatment in the absence of either oxygen and/or alkaline was also investigated on the three different biomasses. Additionally, pre-treatment processes have been tried on wheat straw: steaming and steam explosion for comparison purposes. In all experiments, the yield of hemicellulose solubilisation, enzymatic convertibility of the cellulose fraction, and recovery of both polysaccharides were evaluated. Furthermore, emphasis was also given to the presence of inhibitors in the extracts derived from wet oxidation of wheat straw.

2 Materials and Methods

2.1 Feedstocks

The wheat straws were grown at Risø National Laboratory. Birchwood was kindly supplied by J. Puls, Institute of Wood Science, Hamburg, Germany; willow by K. Hauge Nielsen, Danish Forest and Landscape Research Institute, Vejle, Denmark; and sugar beet pulp by Danisco A/S, Denmark. After harvesting, the feedstocks were dried and ground to 5 mm size. The composition of the untreated feedstocks is shown in **Tables 1** and **2**.

Table 1. *The chemical composition (% dry weight) and the cellulose convertibility of the feedstocks: wheat straws, birchwood, and willow.*

Feedstocks	NCWM (%w/w)	Hemicellulose (%w/w)	Cellulose (%w/w)	Lignin (%w/w)	Ash (%w/w)	Convertibility (%w/w)
Wheat straw 1993	18.8	32.8	38.0	8.9	1.4	12.9
Wheat straw 1994	22.4	29.7	38.9	8.7	0.4	12.3
Wheat straw 1997	25.1	27.9	37.0	8.7	1.4	14.1
Birchwood	14.0	26.4	42.7	16.9	0.1	3.6
Willow	28.8	17.8	34.6	18.8	0.1	13.0

NCWM = Non-cell wall material (pectin, protein etc.)

Table 2. *The chemical composition of sugar beet pulp (% dry weight). Data supplied by Danisco Sugars A/S.*

Pectin (%w/w)	Hemicellulose (%w/w)	Cellulose (%w/w)	Lignin (%w/w)	Protein (%w/w)	Sugar (%w/w)	Fat (%w/w)	Minerals (%w/w)
24.0	31.7	19.7	4.4	10.9	4.4	0.5	4.4

2.2 Pre-treatment

2.2.1 Wet oxidation

Wet-oxidation experiments were carried out in a specially designed loop-reactor (2 L) constructed at Risø National Laboratory with a working volume of 1 L (Bjerre and Sørensen, 1992; Bjerre *et al*., 1996a). The reactor was made of Sandvik Sanicro 28 (27% Cr, 31% Ni, 3.5% Mo, 1% Cu) and mounted on a rack facilitating the control of temperature by immersing the reactor in an appropriate heating and cooling bath. Due to the excellent heat-transfer conditions, the relaxation time was short, which made it suitable also for studies of reaction kinetics.

If alkaline was added, the lignocellulosic material was mixed with Na_2CO_3 and water before adding the oxygen pressure and heating the suspension. Sodium carbonate was chosen because it was cheaper than most other water-soluble alkaline chemicals since lime was not suitable to use in the process. After the pre-treatments, the biomass suspension was filtered to separate the solid cellulose-rich fraction from the liquid hemicellulose-rich fraction. The pH of the liquid fraction was measured and the solid fraction dried and weighed. The composition of both fractions was analysed.

Treatments without oxygen and alkaline were performed in order to identify some of the effect of the added oxygen pressure and sodium carbonate. Hence, one or both of these chemicals were absent in the treatment. The treatment without oxygen was performed by removing the oxygen by blowing nitrogen through the reactor.

2.2.2 Steaming

Preliminary steaming experiments were carried out in Hamburg by Dr. Jürgen Puls, who treated the raw material with saturated steam at different temperatures for 10 minutes (Korte *et al.*, 1991).

2.2.3 Steam explosion

Steam-explosion experiments were carried out in a 5-litre reactor, hence a higher amount of wheat straw (200 g) could be treated per batch compared to the wet-oxidation process (60 g). No catalysts (chemicals) were applied in the process. When the desired steam temperature was reached in the steam generator (A), the steam pressure was measured and led to the reaction vessel (B) (**Figure 3**). When the material had been steamed for the required length of time, the steam pressure was released and the material exploded into the expansion vessel (C). The steam-exploded material was removed from the vessel by water. The resulting suspension was filtered to separate the solid cellulose-rich fraction from the liquid hemicellulose-rich fraction. The pH of the liquid fraction was measured. The solid fractions were dried and weighed. The composition of both fractions was determined.

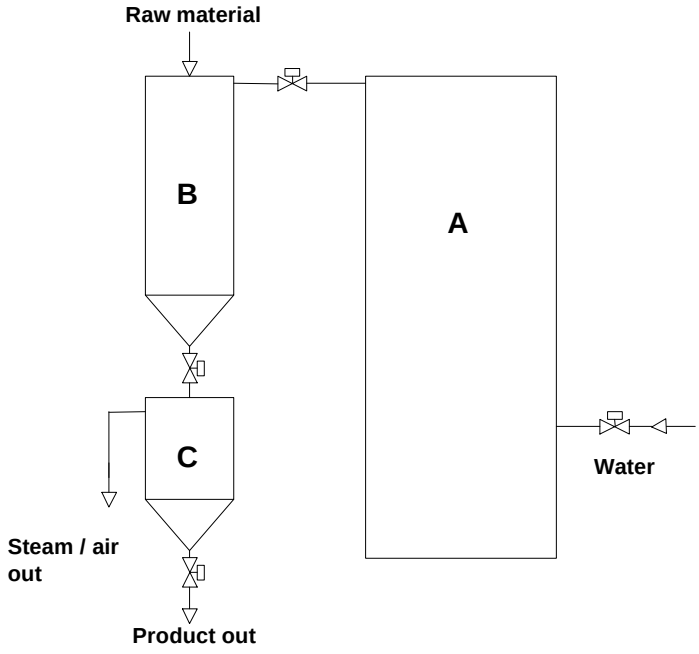

Figure 3. *The steam explosion unit composed of a steam generator (A), a reaction vessel (B) and an expansion vessel (C).*

2.3 Drying

The solid fractions resulting from the pre-treatments were dried *prior* to analysis at 65% relative humidity at 20°C until equilibrium was achieved (after about one week). Stored at these conditions the chemical structure of the solid fraction is stable for up to 10 years (J. Puls, personal communication).

2.4 Acid Hydrolysis

The hemicellulose solubilised in the liquid fraction was quantified by acid hydrolysis with 4% w/v H_2SO_4 at 121°C for 10 minutes. After acid hydrolysis, the solutions were filtered (0.45 μm) in order to remove the water-insoluble residue (Karr and Brink, 1991; Puls, 1993). The hydrolysed samples were purified by combined precipitation and ion exchange (Bjerre *et al.*, 1996b). The sulfate ions were precipitated by $Ba(OH)_2$. After centrifugation (removal of $BaSO_4$) any remaining ions were eliminated by treatment with Amberlite GC-120 (100-200 mesh, H^+) and Dowex 1x4 (50-100 mesh, OH^-) (both Fluka). Samples were purified in duplicate. The recovery of glucose, xylose, and arabinose in the purification procedure was between 82 and 93%. The monosaccharide composition was analysed by HPLC.

2.5 Enzymatic Hydrolysis

The applied enzyme mixture was supplied from VTT Biotechnology and Food Research, Finland, who had combined four commercial enzyme preparations: cellulase (Econase, Alko Ltd., Finland), xylanase (Ecopulp, Alko Ltd., Finland), mannanase (Gamanase, Novo Nordisk A/S, Denmark) and β-glucosidase (Novozym, Novo Nordisk A/S, Denmark) (Schmidt *et al*., 1998). This enzyme mixture was applied for hydrolysis of wet-oxidised wheat straw in order to quantify solubilised hemicellulose. By using enzymatic hydrolysis, the laborious, tedious, and time-consuming sample preparation and purification of the acid hydrolysates *prior* to HPLC analysis could be avoided. The hydrolysis reaction mixture was created by mixing wet-oxidised substrate and the VTT-enzyme mixture with 0.1 M acetate buffer (pH 5.0). To achieve a 1-mL hydrolysis reaction mixture, the total volume of VTT-enzyme mixture and substrate was 250 μL mixed with 750 μL of acetate buffer. The enzyme-substrate-volume-ratio was 0.042, which corresponded to 7,962 nkat xylanase activity per mg dry weight of substrate (Schmidt *et al*., 1998). The hydrolysis mixture was incubated for 24 hours at 45°C in a heated lab shaker (Adolf Kühner AG). The reaction was stopped by heating for 5 minutes at 100°C followed by centrifugation. The supernatant was diluted with deionised water (3:4) and analysed for monosaccharide composition by HPLC.

2.6 Analyses

The developed and existing analytical methods were evaluated and upgraded continually throughout the project.

2.6.1 Fibres

The raw materials and the treated solid fractions were analysed for their concentration of the different fibres: hemicellulose, cellulose and lignin, and the non-cell wall material (NCWM) (water-soluble substances or extractives such as pectin, proteins etc.) by using the gravimetric method of Goering and van Soest (1970). This method is based on the determination of the neutral (NDF) and acid detergent fibre (ADF) followed by a permanganate procedure and incineration. For lignin determination this method avoided interference from protein material and residual waxes present in the wheat straw, which would result in a higher measured lignin content using the Klassons method.

In the NDF analysis, the sample material was boiled in a neutral detergent solution for 1 hour, after which the suspension was filtered quantitatively, washed, dried, and

weighed. The solid fraction was defined as the NDF. In the ADF analysis, the sample material was boiled for 1 hour in an acid detergent solution (0.5 M sulfuric acid). The solid fraction after filtration and drying was defined as the ADF. The lignin content was determined by treating the ADF residual with potassium permanganate and acetate-buffer for 90 minutes (Goering and van Soest, 1970). The solid residual fraction was then incinerated. The hemicellulose content was calculated to be the solid removed by the ADF analysis, the lignin content the solid removed by the permanganate step, and the cellulose content the solid removed by the incineration step. Additionally, the content of NCWM was the solid removed in the NDF analysis. All samples were analysed in duplicate and results were given as a dry matter percentage.

2.6.2 Enzymatic convertibility of cellulose

To evaluate the efficiency of the pre-treatment, the convertibility of the solid cellulose fraction to fermentable glucose was determined using a mixture of two enzymes: the cellulase Celluclast and the *b*-glucosidase Novozym 188 (kindly donated by Novo Nordisk A/S, Bagsværd, Denmark) (Bjerre *et al.*, 1996a). Celluclast: 1.2 g enzyme/mL, corresponding to an activity of 1500 NCU/mL (1 NCU is the amount of enzyme necessary for producing 1 µmole glucose/min from CMC). Novozym 188: 250 CBU/g (1 CBU is the amount of enzyme necessary for producing 1 µmole glucose/min from cellobiose). The developed method was as follows: approximately 100 mg dried filter cake were suspended in 1 mL 0.2 M acetate buffer (pH = 4.8). The enzymes were added and the volume adjusted to 5 mL with deionised water. The activities of the enzymes in the final suspension were: Celluclast: 13.9 NCU/mL and Novozym 188: 0.46 CBU/mL. The mixture was hydrolysed for 24 hr at 50°C. After filtration the concentration of glucose in the filtrate was determined by HPLC. Samples were analysed in duplicate and results given as percentage dry matter of cellulose converted to glucose.

2.6.3 Quantification of sugars

The monosaccharides were quantified by HPLC cation exchange (Aminex HPX-87H column (Biorad)) with a matching pre-column at 63°C (Bjerre *et al.*, 1996b). The sugars were eluted with 0.004 M H_2SO_4 at a flow-rate of 0.6 mL/min and detected by their differential refractometer index (Knauer).

2.6.4 Quantification of furfurals

To investigate the presence of 2-furfural and 5-hydroxymethyl-2-furfural in the pre-treated filtrate an HPLC-method (Nucleosil 5C-18 column) was developed. A linear gradient of methanol (10-90%) in 0.02 M NaH_2PO_4 (pH 3.0) at a flow-rate of 0.7 mL/min was used. The furfurals were detected by a diode array detector at 280 nm using authentic compounds as standard (Bjerre *et al.*, 1996a). Samples were analysed in duplicate.

2.6.5 Quantification of carboxylic acids

The carboxylic acids were determined by ion chromatography (HPICE-AS1 Dionex 4000 i) with 1 mM HCl as eluent at 0.8 mL/min using a combined conductivity and UV (204 nm) detection (Bjerre *et al*., 1996a). Samples were analysed in duplicate.

2.6.6 Quantification of phenolics

Trimethylsilylation of the hydroxyl groups was performed with N,O-Bis-trimethylsilyl-triflouroacetamid (BSTFA) and used to modify the low molecular weight compounds present in pre-treated liquid-fraction such as monomeric phenols and carboxylic acids (Niemelä and Sjöström, 1986). The sample preparation procedure *prior* to GC-MS analysis was performed in two ways: (a) The liquid fraction was adjusted to pH 7 or pH 2 and extracted by solid phase extraction (SPE) with ethyl acetate as eluent.

Volatile phenols such as guaiacol and vanillin were lost due to evaporation procedures (flushing with nitrogen, rotary evaporation, freeze-drying) *prior* to silylation (data not shown). This problem was overcome by drying the extracts from solid phase extraction with Na_2SO_4 *prior* to silylation with BSTFA. This resulted in hardly any losses of volatile phenols, and the total recovery from extraction, drying and silylation were about 90%. The internal standard was chosen to be 1,2,4,5-tetrachlorobenzene, because it is stable and not present in the wet oxidised liquid fraction, also its GC-retention time relative to the analytes was appropriate. The internal standard was added to the eluent ethyl acetate, correcting for volume during the elution of phenols.

The pH 2 extract was dried with Na_2SO_4 and silylated in a mixture of BSTFA-acetonitrile-ethylacetate (volume ration of 1:3:2) at 70°C for 30 minutes. The pH 7 extract was diluted with acetonitrile (b) An aliquot of the liquid fraction was adjusted to pH 7, freeze-dried and silylated as before. Stock solutions of authentic phenolic standards in acetonitrile were stored in darkness at 4°C. Samples of 1-µL were injected (split less) on a GC fused silica capillary column (XTI-5) (30m x 0.25 mm i.d. with a 0.25-µm film) using helium as carrier gas. The GC oven temperature was held at 80°C for 3 min and then increased by 8°C/min to 280°C. The temperature at injector and transfer line was maintained at 250°C. MS spectra were recorded at 70 eV (m/z 30-450). Samples were analysed in duplicates. The MS data was evaluated by using the Varian Saturn GC-MS version 5.2 for calibration and quantification.

2.6.7 COD-determination

The chemical oxygen demand (COD) was analysed using the potassium dichromate method at the Municipal Food and Environmental Control Unit, Køge, Denmark.

2.6.8 TOC-determination

Total organic carbon (TOC) was analysed on a Shimadzu TOC-5000 with IR-detection after combustion at 680ºC (“Pt-catalysed”). Compensation for inorganic carbon was made by acidification.

3 Wet Oxidation Pre-treatment

The wet oxidation process was investigated for fractionation of wheat straw in order to solubilise the hemicellulose fraction, make the cellulose available for enzymatic hydrolysis to glucose, without compromising the recovery of both polysaccharides. Alkaline addition was used during the wet oxidation treatment in order to reduce the production of furfurals and in order to increase the convertibility of the cellulose fraction (Bjerre *et al*., 1996a). The solid and liquid fractions obtained by the process were characterised by their composition.

3.1 Wheat Straw 1993

3.1.1 Factorial design and statistical analysis

The full statistical 2^3-factorial design was used to screen wet-oxidation process parameters (**Table 3**) influencing the reaction products in the solid and liquid fractions (Schmidt and Thomsen, 1998). The performed ANOVA showed significant effects of the reaction temperature and time on the composition of the solid fraction (**Table 4**). Temperature significantly affected the relative amounts of hemicellulose and cellulose, but not that of lignin in the solid fraction. Furthermore, reaction time had a significant effect on the cellulose content, where the two factors (temperature and time) were interrelated. The convertibility to glucose of the enriched cellulose fraction was significantly affected by the reaction temperature and time as well as by the oxygen pressure. The reaction temperature, the most important parameter, had a significant effect on the composition of the liquid fraction (COD-value and concentration of xylose and arabinose (after acid hydrolysis)) (**Table 4**).

Table 3. *The statistical 2^3-factorial design for wet oxidation of wheat straw 1993 (*60 g/L straw, 6.5 g/L Na_2CO_3*). (Schmidt and Thomsen, 1998).*

Factor	High level	Low level
Oxygen pressure	12 bar	6 bar
Reaction temperature	185°C	150°C
Reaction time	15 min	5 min

Table 4. *The effects of the factors evaluated by the ANOVA for wet oxidation of wheat straw 1993. (Schmidt and Thomsen, 1998).*

Reaction products	Oxygen pressure	Reaction temperature	Reaction time
Solid fraction	ns	**	ns
Hemicellulose	ns	**	ns
Lignin	ns	ns	ns
Cellulose[a]	ns	*	*
Convertible cellulose[b]	*	*/**	**
COD	ns	***	ns
Glucose	ns	ns	ns
Xylose	ns	***	*
Arabinose	ns	***	ns
pH	ns	*	*

ns, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$

a: reaction temperature and time were interrelated

b: oxygen pressure and reaction temperature were interrelated, and reaction temperature and time were interrelated

The experiments were analysed in more details by performing a standardised Principal Component Analysis (PCA) using the Unscrambler software (CAMO A/S,

Norway) with full cross validation (Schmidt and Thomsen, 1998). The following effects were observed in PCA (Schmidt and Thomsen, 1998), which were not significant in ANOVA: lignin content varied systematically with reaction temperature and time; the reaction time at 6 bar oxygen pressure showed systematically variation in glucose concentration; and the expected effect of oxygen pressure on the lignin degradation was observed. This was in accordance with McGinnis *et al.* (1983). A high relative content of lignin in the fibres was associated with low oxygen pressure and was negatively correlated with the content of hemicellulose in the solid fraction, which was associated with a higher oxygen pressure.

At higher reaction temperature and longer reaction time, the solid fibre fraction was enriched in cellulose (Schmidt and Thomsen, 1998). This was explained by lignin and hemicellulose removal due to degradation and/or solubilisation (**Table 5**), in accordance with results of McGinnis *et al.* (1983). The cellulose convertibility increased up to 66% w/w by wet-oxidation treatment compared to untreated wheat straw (13% w/w). However, previously even higher conversion was found by wet oxidation (85% w/w), where process conditions of relatively higher level of oxygen and Na_2CO_3 addition were used due to the use of lower straw concentrations (Bjerre *et al.*, 1996a). The lower convertibility was probably due to a higher lignin concentration in treated fibres (8% w/w) compared to the previous study (4% w/w). Furthermore, the amount of solubilised hemicellulose (measured as pentoses after acid hydrolysis) in the liquid fraction increased at higher reaction temperature and longer reaction time (**Table 5**). Wet oxidation condition of 185°C, 15 minutes and 12-bar oxygen pressure gave the best fractionation and the highest amount of solubilised hemicellulose and convertible cellulose in the statistical design.

Table 5. *The composition of the solid (% dry weight) and liquid fractions from wet-oxidised wheat straw 1993 in the 2^3-factorial design. (Schmidt and Thomsen, 1998).*

Initial or Reaction Products		Feedstock[a]	150°C[b] 6 bar 5 min	150°C 6 bar 15 min	150°C 12 bar 5 min	150°C 12 bar 15 min	185°C 6 bar 5 min	185°C 6 bar 15 min	185°C 12 bar 5 min	185°C 12 bar 15 min
Solid fraction	(g)	60	43.2	42.9	45.2	42.4	35.5	29.1	35.5	30.6
Hemicellulose	(%w/w)	32.8	27.8	27.2	30.4	26.9	16.5	10.1	19.2	12.2
Lignin	(%w/w)	8.9	12.5	12.5	11.5	10.9	11.3	8.7	9.4	7.7
Cellulose	(%w/w)	38.0	48.8	50.0	49.1	51.6	61.6	69.4	60.0	68.2
Convertible cellulose[c]	(%w/w)	12.9	32.0	37.4	30.2	35.8	46.4	65.4	47.4	66.3
Liquid fraction										
COD	(mg O_2/L)	-	16000	18000	15000	19000	25000	28000	27000	29000
Glucose	(g/L)	-	0.91	1.15	0.86	0.86	0.98	0.57	0.95	1.07
Xylose	(g/L)	-	1.48	2.26	1.30	1.88	5.44	7.16	5.34	7.39
Arabinose	(g/L)	-	0.38	0.58	0.31	0.53	1.08	1.38	1.22	1.35
pH		10.5[d]	7.8	7.2	7.8	7.2	6.8	6.4	6.9	5.9

a: Feedstock: 18.8% w/w non-cell wall materials (protein, pectin etc.) and 1.4% w/w ash.

b: Wet oxidation conditions, *i.e.* temperature, O_2-pressure, reaction time. Constant parameters: 60 g/L wheat straw and 6.5 g/L Na_2CO_3.

c: % of cellulose in solid fraction

d: pH-value of reaction mixture before wet oxidation

3.1.2 Effect of reaction temperature

Studying the effect of reaction temperature on the hemicellulose solubilisation, a lower hemicellulose concentration was observed at 200°C compared to that at 185°C (Schmidt and Thomsen, 1998). This might be due to a higher degradation of this component at 200ºC, since the same percentage of the original hemicellulose amount was removed from the solid fraction (80% w/w) (**Figure 4**). In the treated fibres, more than 50% w/w of the original lignin was removed at temperatures above 185°C, which has previously been found to be the optimal reduction in original lignin in wheat straw for cellulose hydrolysis (Binder *et al*., 1980). On raising the temperature to 200°C, the thermal degradation of the cellulose began (**Figure 4**), in accordance with McGinnis *et al*. (1983).

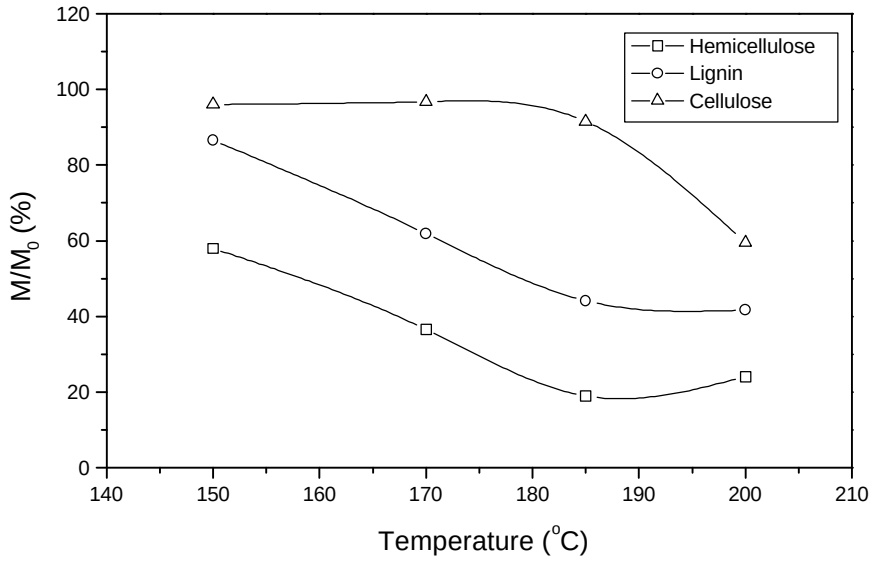

Figure 4. *The relative characteristics (M/M_0) of the solid fraction (hemicellulose, lignin and cellulose) obtained by the wet oxidation of wheat straw 1993 as a function of the reaction temperature (60 g/L straw, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material. (Schmidt and Thomsen, 1998).*

3.1.2.1 Mass balances

The recovery was calculated for cellulose and hemicellulose to estimate their losses during wet oxidation at different temperatures (**Table 6**). The calculation was based on mass balances, as shown below for cellulose at 185°C based on 60-g wheat straw:

$$Recovery\ of\ cellulose\ (\%) = \frac{cellulose\ out\ (treated\ straw) + glucose\ produced\ (liquid) \cdot \frac{162}{180}}{cellulose\ in\ (untreated\ straw)} \cdot 100\%$$

$$= \frac{20.9g + 1.1g \cdot \frac{162}{180}}{22.8g} \cdot 100\% = 95.8\%$$

In wet oxidation, mainly the hemicellulose was converted and/or degraded (**Table 6**). About 60% of the original hemicellulose could be recovered, which is similar to the results found with steaming (Heitz *et al*., 1991). The more severe pre-treatment

(higher temperature) the lower the recoveries. At temperatures up to 185°C, the recovery for cellulose in the solid fraction was 100% (**Table 6** and **Figure 4**). Above 185°C, the recovery and convertibility decreased as cellulose was removed from the solid fraction and broken down. An overall recovery of 80% carbohydrates was obtained by wet oxidation. The results showed that wet oxidation was a balance between solubilisation and degradation. The longer the reaction time and higher temperature the more hemicellulose was solubilised, but its degradation also increased thereby decreasing its recovery.

Table 6. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) from wheat straw 1993 as a function of the wet oxidation temperature (60 g/L straw, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Thomsen, 1998).*

Temperature (°C)	Hemicellulose (%)	Cellulose (%)	Overall Recovery (%)
150	69	99	85
170	60	100	80
185	58	96	76
200	47	62	54

In order to verify the presence of any unidentified components in the liquid fraction, a mass balance was calculated based on the COD-content. The COD-factors used were determined for each component based on its reaction with oxygen giving carbon dioxide and water. The COD-factors and COD-contributions for all identified components were calculated and summarised (**Table 7**). The calculated COD-content could account for only about half of the measured COD-value. This was very surprising. There could be various reasons for this finding. Several unidentified components were possibly present, such as other acids and possible some lignin degradation products, in particularly since 50-70% of the lignin in wheat straw was degraded by wet oxidation. Another reason could be that the analytical method for COD determination is difficult to perform in particular for inhomogenic mixtures *e.g.* precipitated hemicellulose.

Table 7. *The measured COD-content compared to the calculated COD-content based on identified sugars and carboxylic acid components in the liquid fraction after wet oxidation of wheat straw 1993 (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Thomsen, 1998).*

Identified component	Concentration (g/L)	COD-factor (mg O_2/g)	COD-contribution (mg O_2/L)
Glucose	1.07	1067	1144
Xylose	7.39	1067	7885
Arabinose	1.35	1067	1439
Formic acid	1.53	348	531
Acetic acid	2.10	1067	2245
Glycolic acid	1.12	632	707
Oxalic acid	0.73	178	130
Maleic acid	0.17	716	122
Σ COD-contributions			14203
Measured COD-content			29000

COD factor = the amount of O_2 needed to oxidise 1 g of the identified component to CO_2 and H_2O

3.1.3 Rate of fractionation

The actual *rate of fractionation* of the solid fraction was investigated at 185°C and 200°C. The reaction kinetics were simplified by considering the fractionation as hydrolysis and assuming that hemicellulose, lignin, and cellulose only reacted with oxygen in the aqueous solution. The reactions were considered to be irreversible and follow a second order:

$$\text{Hemicellulose (H) + Oxygen (O)} \xrightarrow{k_1} \text{Products (P)}$$
$$\text{Lignin (L) + Oxygen (O)} \xrightarrow{k_2} \text{Products (P)}$$
$$\text{Cellulose (C) + Oxygen (O)} \xrightarrow{k_3} \text{Products (P)}$$

For hemicellulose at the concentration of C_H, the reaction rate (r_H) could be expressed as:

$$-r_H = -\frac{dC_H}{dt} = -\frac{dC_O}{dt} = k_1 \cdot C_H \cdot C_O \qquad (1)$$

If oxygen is in large surplus, the concentration of oxygen (C_O) can be considered to be constant giving the following equation:

$$-r_H = -\frac{dC_H}{dt} = k_1' \cdot C_H \qquad (2)$$

The reaction is now of pseudo first order and can be described as:

$$-\ln\frac{C_H}{C_{H0}} = k_1' \cdot t + c \qquad (3)$$

Where k_1' is the rate constant (min^{-1}), t is the reaction time (min), C_{H0} the initial concentration of the hemicellulose (g/L), and c a constant. Similar equations can be derived for lignin and cellulose. The kinetics were determined by plotting -ln C/C_0 as a function of the reaction time. The rate constant was given by the linear regression coefficient.

In **Table 8** the rate constants for the removal of hemicellulose, lignin, and cellulose are shown after wet oxidation at 185°C. The reaction kinetics for the components all fitted well with a pseudo first order kinetic with high correlation coefficients ($r > 0.9$). Previously, this has been obtained for simplified reactions of wet oxidation with low molecular weight compounds (Bjerre and Sørensen, 1992). The best fit was achieved for hemicellulose, then lignin and cellulose (**Table 8**). The rate constants for cellulose were about 6-8 times lower than for hemicellulose and lignin, which confirms that the cellulose was the most stable component during wet oxidation conditions. Furthermore, the rate constants were in agreement with findings of Fengel and Wegener (1989). At 200°C the data did not fit a pseudo first order kinetic (data not shown) probably due to the coating of cellulose at high temperature.

Table 8. *The rate constants for* ***removal*** *of hemicellulose, lignin and cellulose from wheat straw 1993 solid fraction by wet oxidation (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2) according to equation (3). (Schmidt and Thomsen, 1998).*

Component	k' (min^{-1})	c	r
Hemicellulose	0.058 ± 0.004[a]	0.68 ± 0.07	0.99
Lignin	0.042 ± 0.009	0.22 ± 0.16	0.94
Cellulose	0.007 ± 0.002	-0.01 ± 0.03	0.92

a: Mean value and standard derivation

3.1.4 Effect of oxygen and alkaline

The effect of alkaline addition to the wet oxidation process was mainly seen in the higher removal of lignin from the solid fraction (**Figure 5**). A maximum removal of lignin was found when treating the straw with 13 g/L sodium carbonate (80%). However, the maximum cellulose content was already obtained when adding 2-4 g/L sodium carbonate due to a high removal of both hemicellulose and NCWM at this treatment.

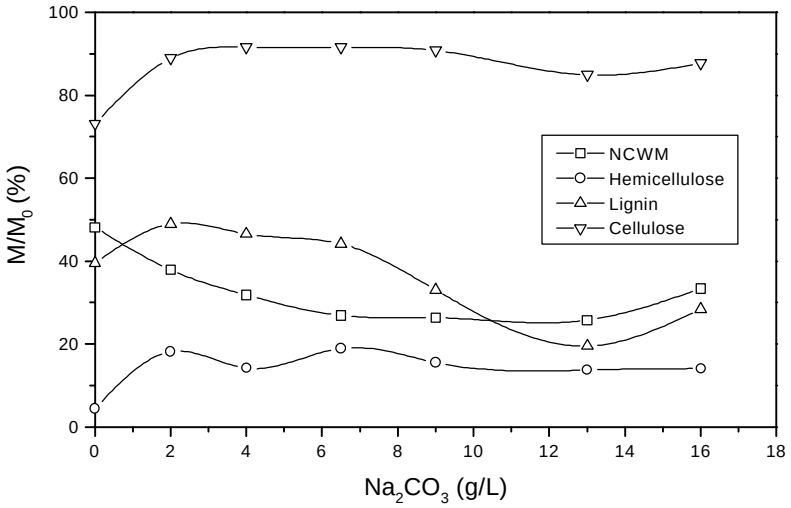

Figure 5. *The relative characteristics (M/M_0) of the solid fraction (non-cell wall material (NCWM), hemicellulose, lignin and cellulose) obtained by the wet oxidation of wheat straw 1993 (60 g/L straw, 185°C, 12 bar O_2, 15 minutes) as a function of the sodium carbonate concentration. M: Mass of component in solid fraction. M_0: Mass of component in starting material. (Schmidt and Thomsen, 1999).*

The enzymatic hydrolysis of pre-treated fibres was dependent of both alkaline and oxygen addition in the process (**Figure 6**). When treating with water alone (no oxygen/alkaline) up to 38% of the cellulose fraction was convertible by the enzyme treatment after a prolonged hydrolysis time (above 24 hours). With alkaline addition in the process but no oxygen present the convertibility increased to 60%. The highest convertibility was achieved by wet oxidation with (65%) and in particular without additional alkaline in the process, where the maximum convertibility was almost 80%. Both experiments resulted in low lignin contents in the treated solid fraction. With hydrolysis times shorter than 24 hours no significant difference was observed between alkaline wet oxidation and wet oxidation. For all treatments, most of the cellulose that was hydrolysed in 96 hours was hydrolysed within the first 18-24 hours.

The importance of the lignin content for the enzymatic convertibility was revealed by plotting the convertibility as a function of the lignin content for the different reaction temperatures (**Figure 7**). The results were plotted independent of other reaction conditions. The tendency was that a high convertibility was accomplished by low lignin content in the treated fibre fraction. In a few experiments carried out at high temperature a high convertibility was found also in fibres with an average lignin content.

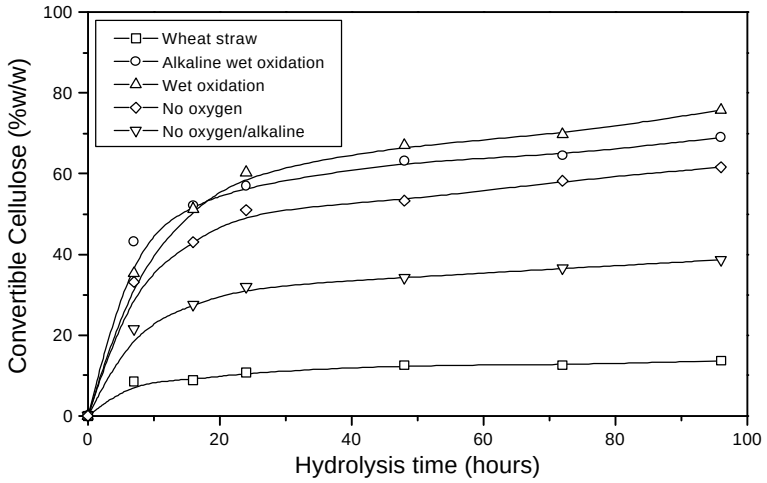

Figure 6. *The enzymatic convertibility of the cellulose present in the solid fraction after treatment of wheat straw 1993 as a function of the hydrolysis time and pre-treatment conditions (Alkaline wet oxidation: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Thomsen, 1999).*

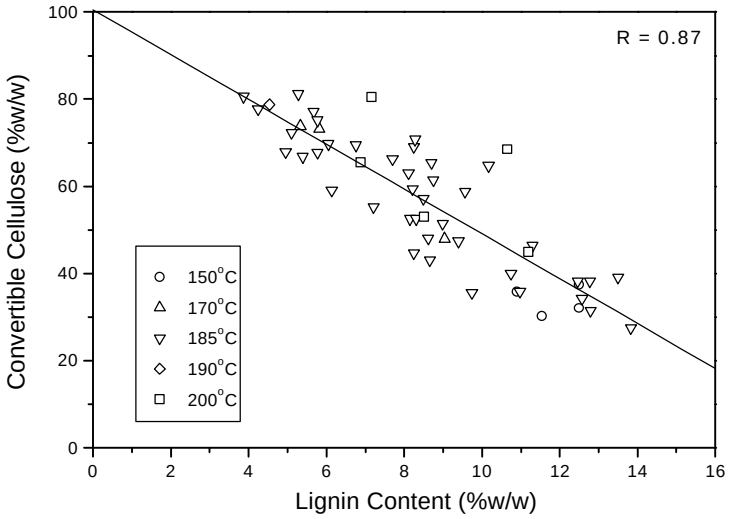

Figure 7. *The relationship between the enzymatic convertibility of the cellulose and the lignin content in the solid fractions after wet oxidation and hydrothermal treatment of wheat straw 1993 at low temperatures (150-200°C). (Schmidt and Thomsen, 1999).*

The recovery of hemicellulose and cellulose were also dependent of the additions of chemicals (**Table 9**). By addition of alkaline the recoveries were increased during hydrothermal treatment and particular during wet oxidation. For wet oxidation in the

absence of alkaline low recoveries for hemicellulose and cellulose were obtained. The presence of alkaline might give higher recoveries due to lower degradation of sugars into other products *e.g.* furfurals.

Table 9. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) in pre-treatment of wheat straw 1993 (Alkaline wet oxidation: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Thomsen, 1999).*

Pre-treatment	pH	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
Alkaline wet oxidation	5.9	58	96	78
Wet oxidation	3.0	48	78	64
No oxygen	7.0	61	97	80
No oxygen/alkaline	4.9	59	97	79

Pentoses like xylose will degrade to 2-furfural mainly at low pH as illustrated in **Figure 8**. The tendency (r = 0.76) is that low final pH-values will result in higher 2-furfural formation. Lower concentration of 2-furfural was found in pretreatment with alkaline present in the process (**Table 10**).

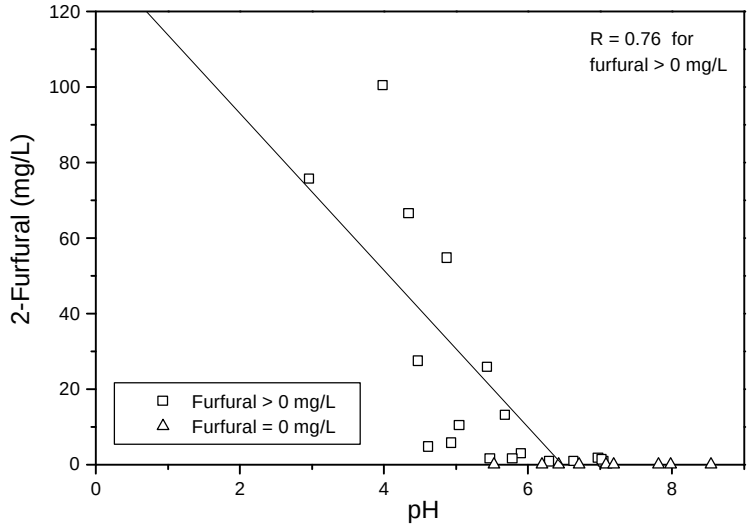

Figure 8. *The relationship between the formation of 2-furfural and the final-pH in the liquid fraction after wet oxidation of wheat straw 1993 at 185°C. (Schmidt and Thomsen, 1999).*

Table 10. *Formation of 2-furfural from pre-treatment of wheat straw 1993 (Alkaline wet oxidation: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Thomsen, 1999).*

Pre-treatment	pH	2-Furfural (mg/L)
Alkaline wet oxidation	5.9	3
Wet oxidation	3.0	76
No oxygen	7.0	2
No oxygen/alkaline	4.9	55

3.2 Wheat Straw 1994

As the wheat straw from 1993, used so far for pre-treatment, had been consumed, a change to another wheat straw batch (wheat straw 1994) was necessary. Wheat straw from 1994 behaved differently in wet oxidation than the straw from 1993. This is probably due to the fact that biological material varies from year to year due also to variation in the weather. Hence, wet oxidation conditions had to be investigated to ensure a reasonable substrate for fermentation purposes.

3.2.1 Effect of temperature

For wheat straw 1993, temperature was a very important wet oxidation parameter (Schmidt and Thomsen, 1998), hence, the effect of temperature on wet oxidation of wheat straw 1994 was investigated. No clear temperature optimum could be observed for the chemical composition of the solid fraction (**Figure 9**), in contrast to the case for straw 1993 (Schmidt and Thomsen, 1998). A very high convertibility of the cellulose in the solid fraction was found for straw 1994. At 6-bar oxygen there was a clear optimum at 190°C giving a conversion of cellulose to glucose of more than 90%. At 12-bar oxygen, 210°C was needed for a total conversion of cellulose to glucose, however, at this temperature the cellulose content decreased presumably due to decomposition.

A higher wet oxidation temperature was necessary for solubilisation of the hemicellulose from wheat straw 1994 (195°C) (**Figure 10**) compared to straw 1993 (185°C) at the same process conditions (Schmidt and Thomsen, 1998). A temperature of 195 and 190°C was needed at respectively 12 and 6 bar oxygen pressure to achieve the highest amount of solubilised hemicellulose. The maximum concentration of total sugars was the same (approximately 8 g/L) for the two oxygen pressures. However, the sugar levels were still significantly lower than for wheat straw from 1993 (Schmidt and Thomsen, 1998).

As seen before, several different carboxylic acids were formed during wet oxidation (**Table 11**), but the level was slightly lower for wheat straw 1994 than for straw 1993 (Bjerre and Schmidt, 1997). No iso-butyric acid was formed in any of the applied temperatures. The dominant acid was acetic acid as for wheat straw 1993. As temperature increased the formation of glycolic acid increased whereas the formation of formic acid decreased. The total organic carbon (TOC) was highest at temperatures between 195-200ºC (**Table 11**). At 200ºC the amount of carboxylic acid and solubilised hemicellulose was lower than at 195ºC, hence, other compounds must contribute to the TOC at 200ºC.

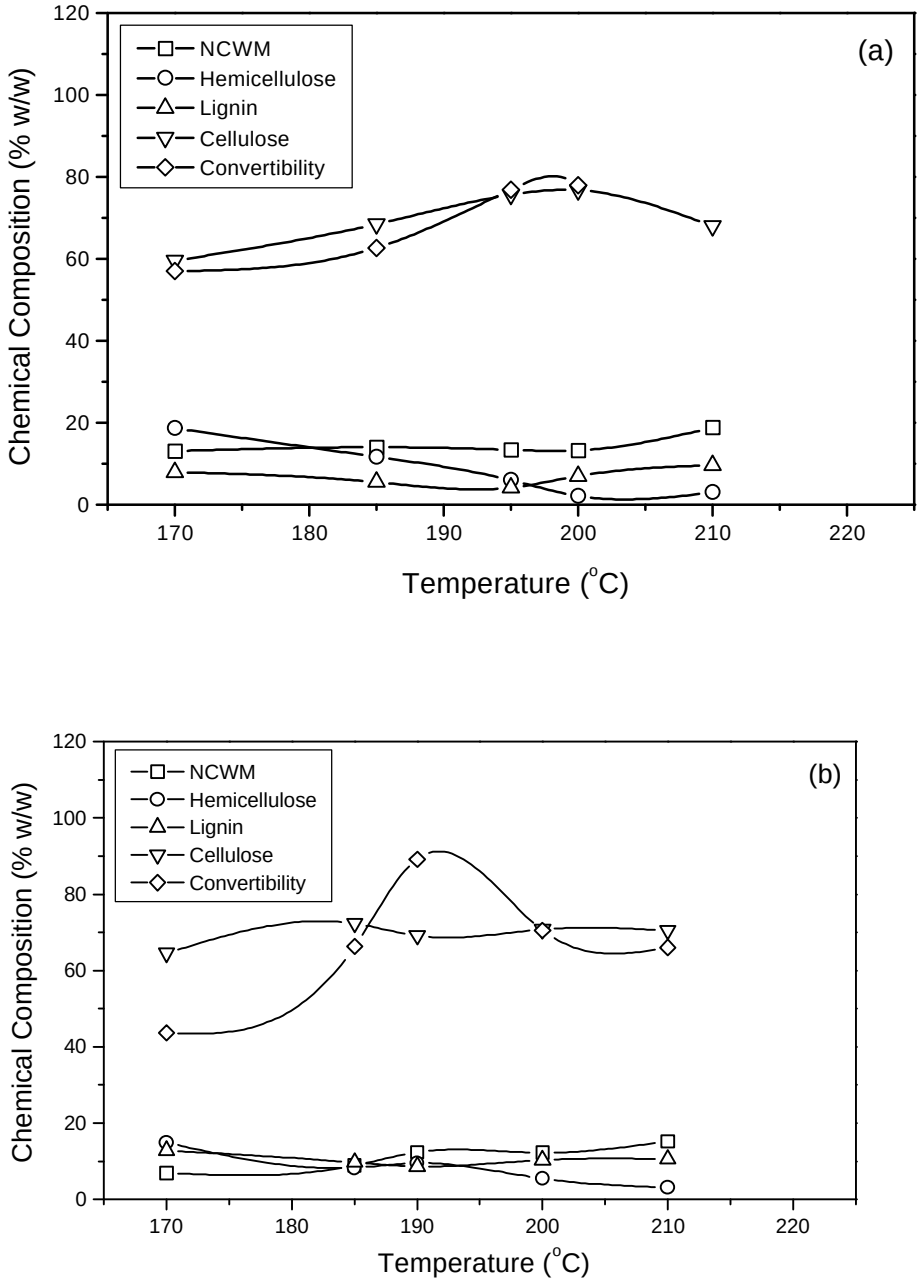

Figure 9. *Effect of wet oxidation temperature on the chemical composition (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) (% dry weight) and the cellulose convertibility of the solid fraction from wheat straw 1994 at 2 different oxygen pressures (60 g/L straw, 6.5 g/L Na_2CO_3, 15 minutes).* ***a)*** *12 bar O_2.* ***b)*** *6 bar O_2.*

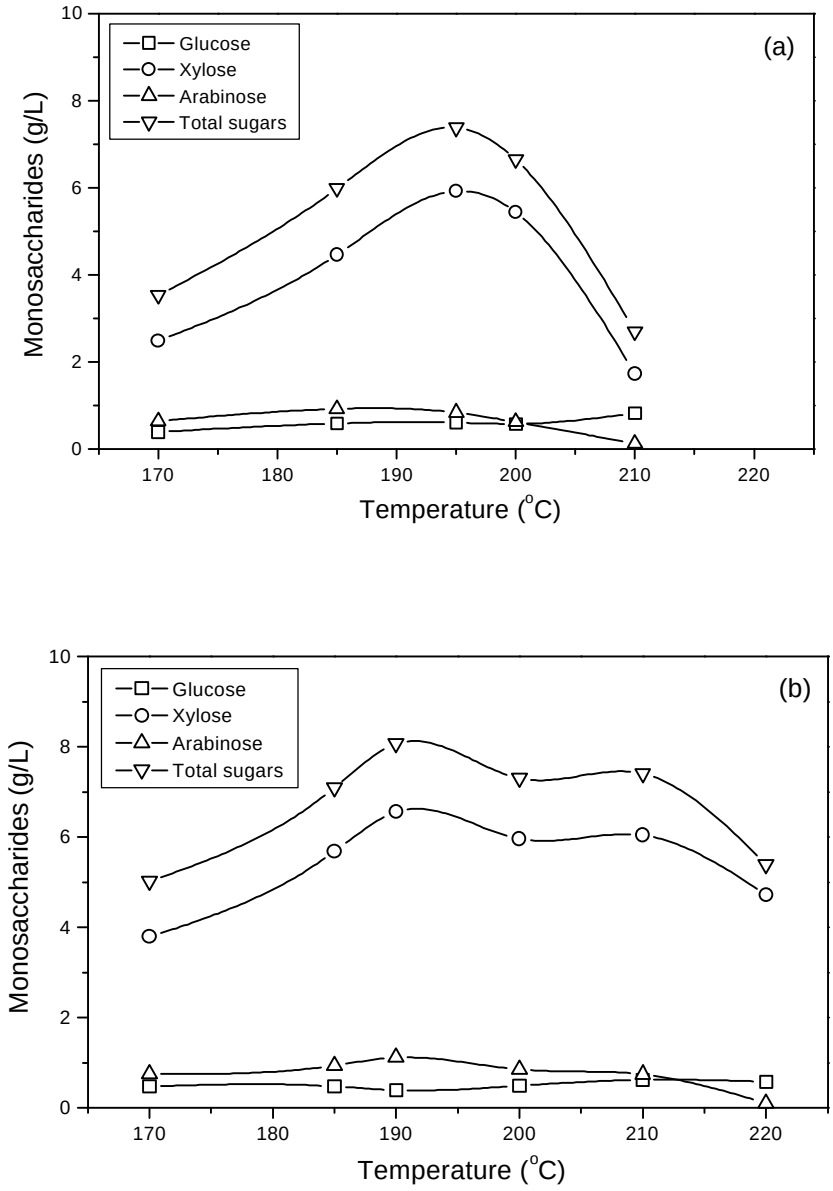

Figure 10. *Effect of wet oxidation temperature on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from wheat straw 1994 at two different oxygen pressures (60 g/L straw, 6.5 g/L Na_2CO_3, 15 minutes).* ***a)*** *12 bar O_2.* ***b)*** *6 bar O_2.*

Table 11. *Effect of wet oxidation temperature on the formation of carboxylic acids and total organic carbon (TOC) from wheat straw 1994 (60 g/L straw, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Temperature (°C)	TOC (g C/L)	Formic (g/L)	Acetic (g/L)	Glycolic (g/L)	Malic (g/L)	Oxalic (g/L)	Total (g/L)
185	9.9	1.09	2.46	0.74	0.37	0.011	4.67
195	11.4	1.35	2.58	1.20	0.31	0.015	5.46
200	10.7	0.27	2.14	1.22	0.25	0.019	3.90
210	8.9	0.29	2.84	2.01	0.26	0.013	5.40

The level of produced 2-furfural for wheat straw 1994 was similar to that for wheat straw 1993 (**Table 12**). However, treatment at 12-bar oxygen resulted in significantly higher concentration of 2-furfural compared with treatment at 6-bar oxygen. At 12-bar oxygen, more carboxylic acids (and CO_2) were found due to the more severe oxidation conditions. Therefore, a decrease in the pH-value was obtained in the reaction mixture, and hence, an increased formation of 2-furfural in accordance with **Figure 8**.

Table 12. *Effect of wet oxidation temperature on the formation of 2-furfural (mg/L) from wheat straw 1994 at two different oxygen pressures (60 g/L straw, 6.5 g/L Na_2CO_3, 15 minutes).*

Temperature	12 bar Oxygen		6 bar Oxygen	
(°C)	pH	2-Furfural	pH	2-Furfural
170	6.8	0	5.6	0
185	6.4	0	6.8	0
190	-[a]	-[a]	6.9	0.2
195	5.2	9.5	6.5	-[b]
200	4.9	29.7	5.7	1.5
210	4.6	108.3	5.6	8.6
220	-[a]	-[a]	5.1	15.7

a: wet oxidation experiment not performed
b: analysis not performed

3.2.2 Effect of reaction time

The reaction time was previously (Schmidt and Thomsen, 1998) found to have a significant effect on the degree of fractionation during the wet-oxidation process. For wheat straw 1993 the optimum reaction time was 15 minutes with a convertibility of the cellulose fraction of 67%. For wheat straw 1994, the convertibility of the cellulose had a very clear optimum after just 5 minutes reaction time (**Figure 11**), where more than 95% of the cellulose could be converted to glucose for fermentation purposes. This was the maximum conversion yield obtained during our research with wet oxidation.

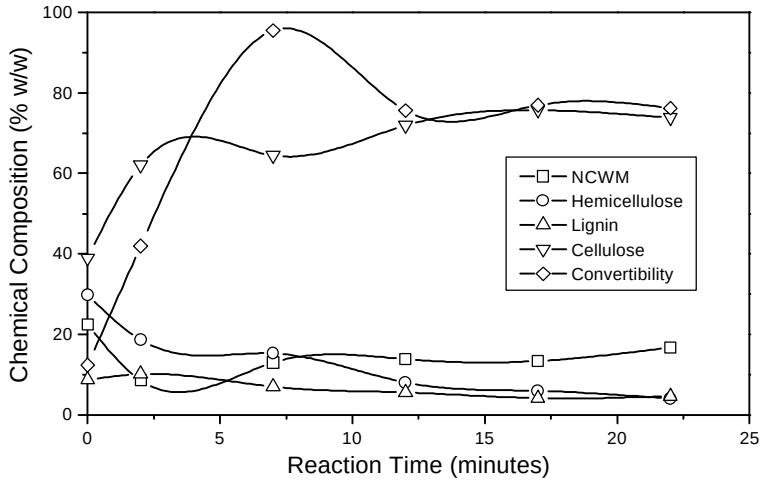

Figure 11. *Effect of wet oxidation reaction time on the chemical composition (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) (% dry weight) and cellulose convertibility of the solid fraction from wheat straw 1994 (60 g/L straw, 195°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

This high conversion might be partly explained by the efficient removal of lignin, hemicellulose and non-cell wall material (NCWM) after just a short treatment time (5 minutes) (**Figure 12**) resulting in an enriched cellulose fraction. A reaction time of 5 minutes seemed long enough for maximal solubilisation of hemicellulose (**Figure 13**). However, for wheat straw 1994, the amount of solubilised hemicellulose was still lower than what had been found for wheat straw 1993.

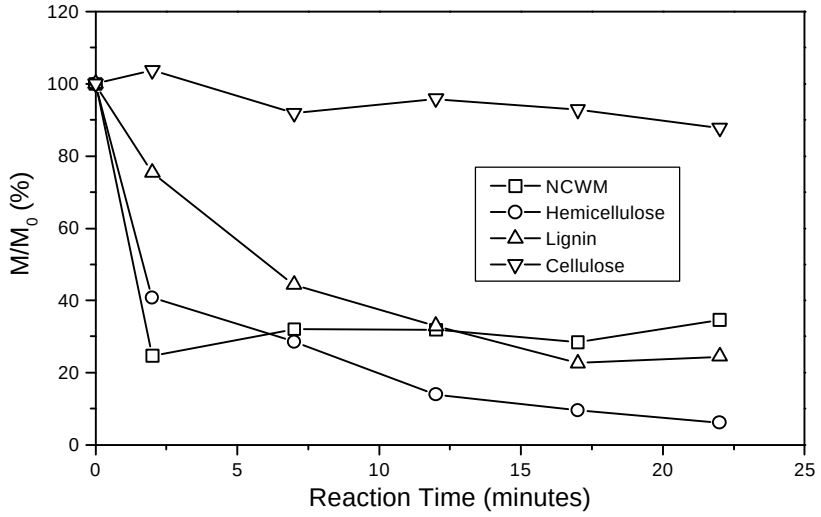

Figure 12. *Effect of wet oxidation reaction time on removal of non-cell wall material (NCWM), hemicellulose, lignin, and cellulose from the solid fraction (M/M_0) for wheat straw 1994 (60 g/L straw, 195°C, 6.5 g/L Na_2CO_3, 12 bar O_2). M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

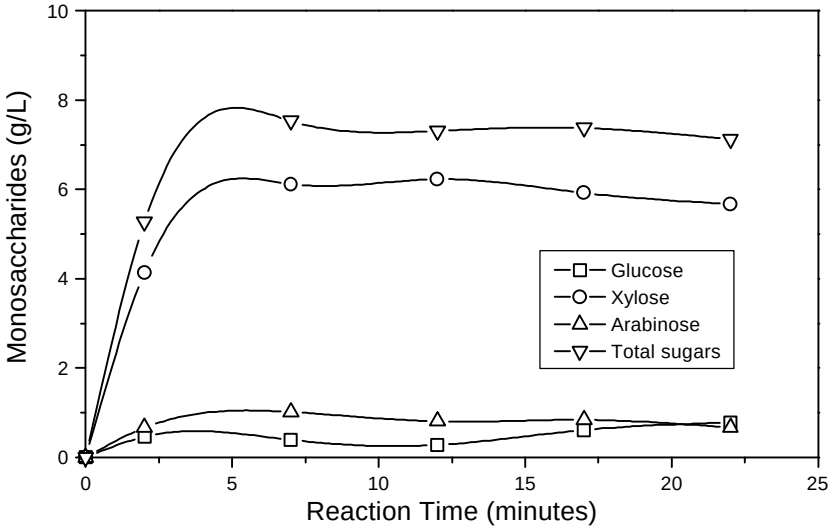

Figure 13. *Effect of wet oxidation reaction time on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from wheat straw 1994 (60 g/L straw, 195°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

The recovery of the two polysaccharides were calculated (**Table 13**). In general, very high recoveries were found for the short treatment time even for hemicellulose. The recovery decreased as the treatment time increased. When the wheat straw 1994

was treated for longer than 5 minutes the recovery for hemicellulose dropped to less than 50%. These were the same conditions that gave the very high cellulose convertibility (**Figure 12**).

Table 13. *Effect of wet oxidation reaction time on the calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) after pre-treatment of wheat straw 1994 (60 g/L straw, 195°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

Reaction Time[a] (minutes)	2	7	12	17	22
Hemicellulose (%)	64.4	63.8	48.7	42.9	38.2
Cellulose (%)	105.2	93.1	96.7	94.8	90.3
Total Polysaccharides (%)	87.6	80.4	76.0	72.3	67.7

a: Reaction times are included the 2 minutes heating and cooling times.

The level of formed furfurals from wet oxidation of wheat straw 1994 was similar to that of wheat straw 1993 (**Table 14**). The amount increased with time, as did the total organic carbon (TOC). This was in agreement with the lower recovery (**Table 13**) as a function of time. Carboxylic acids have only been analysed at 195°C for the 12 minutes reaction time (**Table 11**).

Table 14. *Effect of wet oxidation reaction time on formation of furfurals (2-furfural and 5-hydroxymethyl-2-furfural (HMF)) and total organic carbon (TOC) from wheat straw 1994 (60 g/L straw, 195°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

Reaction Time[a] (minutes)	2	7	12	17	22
pH	7.1	6.7	5.7	5.2	4.9
2-Furfural (mg/L)	2.6	0	1.1	9.5	29.9
HMF (mg/L)	0.6	0.8	0.1	0.3	1.4
TOC (g C/L)	8.3	10.1	10.9	11.4	10.6

a: Reaction times are included the 2 minutes heating and cooling times.

3.2.3 Effect of oxygen and alkaline

The effect of oxygen pressure and alkaline addition during the pre-treatment process was also investigated for wheat straw 1994. The composition of the straw changed dramatically during the pre-treatment (**Figure 14**). Very high cellulose convertibility was obtained at three pre-treatment conditions: wet oxidation with and without alkaline and alkaline hydrolysis (no oxygen). This could be related to the low lignin content in the solid fraction, in particular for the alkaline wet oxidation where extremely high convertibility was achieved (90%).

The solubilisation of the hemicellulose showed that the alkaline wet oxidation gave relative low level of hemicellulose (6 g/L) (**Figure 15**). On the other hand, wet oxidation without alkaline addition gave the highest amount of solubilised hemicellulose (about 9 g/L). Surprisingly, even adding only water (no oxygen/alkaline) gave a higher amount of hemicellulose than alkaline wet oxidation. Hence, it is difficult to obtain both high cellulose convertibility and high amount of solubilised hemicellulose.

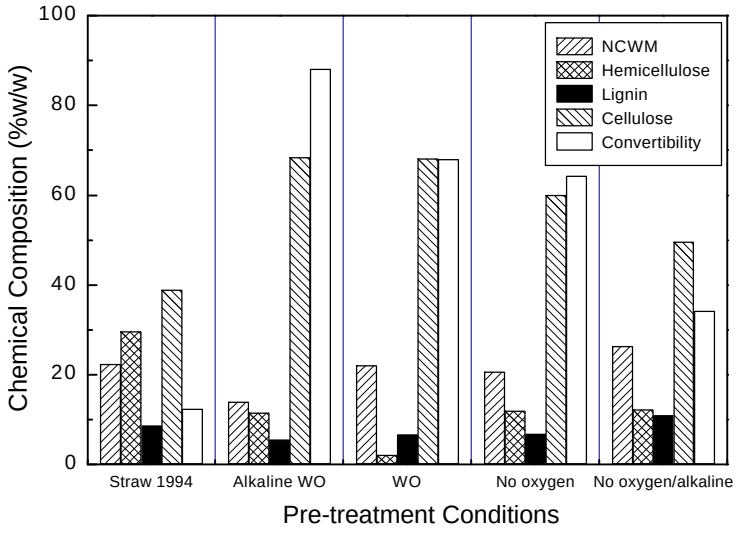

Figure 14. *Chemical composition (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) (% dry weight) and the enzymatic convertibility of the cellulose present in the solid fraction from wheat straw 1994 at different pre-treatment conditions (Alkaline WO: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). WO: Wet oxidation.*

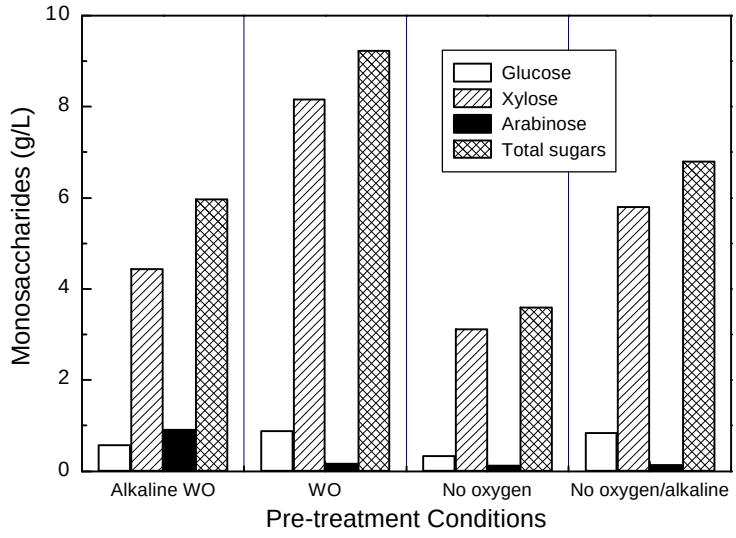

Figure 15. *Hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from wheat straw 1994 at different pre-treatment conditions (Alkaline WO: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). WO: Wet oxidation.*

The recovery of the two polysaccharides was calculated for wet oxidation in presence and absence of alkaline (**Table 15**). The recovery for hemicellulose was very low (less than 50%). In general, the alkaline wet oxidation gave higher recoveries than when alkaline was absent giving a recovery close to 100% for cellulose. This is in accordance with other observations from wet oxidation studies. Most compounds are most reactive during neutral to acid conditions and more stable during alkaline conditions (Thomsen, 1998).

Table 15. *Effect of pre-treatment conditions on the calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) from wheat straw 1994 (Alkaline wet oxidation: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Pre-treatment	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
Alkaline wet oxidation	45.4	99.2	75.9
Wet oxidation	38.8	86.8	66.0

The formation of the sugar degradation products, furfurals, was strongly influenced by the addition of oxygen pressure and alkaline (**Table 16**) as observed previously for straw 1993 (Schmidt and Thomsen, 1999). The alkaline addition resulted in no formation of any measurable amount of furfurals. Wet oxidation in the absence of alkaline gave the highest amount of furfurals due to formation of acidic compounds and hence low final-pH. However, this treatment still resulted in the highest amount of solubilised hemicellulose.

Table 16. *Formation of furfurals from wheat straw 1994 at different pre-treatment conditions (Alkaline wet oxidation: 60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Pre-treatment	pH	2-Furfural (mg/L)	5-Hydroxymethyl-2-furfural (mg/L)
Alkaline wet oxidation	6.4	<1	<1
Wet oxidation	3.1	183	13.5
No oxygen	7.3	<1	<1
No oxygen/alkaline	4.2	75.7	4.15

3.3 Wheat Straw 1997

A reduced statistical 2^4-factorial design was used in order to determine the importance of different wet oxidation parameters for optimisation of the wet-oxidation process of wheat straw 1997. Four factors were selected: temperature, sodium carbonate concentration, oxygen pressure, and reaction time at 2 levels (**Table 17**) yielding 8 different experiments. The levels were selected based on results for wheat straw 1993 and 1994. In the design the concentrations of wheat straw was kept constant. The order in which the 8 experiments were carried out was randomised. The same operator ran all wet oxidations within one week in order to minimise block effects.

Table 17. *The statistical $½^x2^4$-factorial design for wet oxidation applied for wheat straw 1997 (60 g/L straw).*

Factor	Parameter	High level	Low level	Units
A	Temperature	195	185	°C
B	Na_2CO_3	6.5	2	g/L
C	Oxygen	12	6	bar
D	Reaction Time	15	10	minutes

The levels of the parameters were chosen based on previous studies on wheat straw 1993 and 1994 (Schmidt and Thomsen, 1998; this report). The design chosen gave the wet oxidation conditions illustrated in **Table 18**.

Table 18. *Applied wet oxidation conditions of wheat straw 1997. (Klinke et al., 1998).*

Conditions[a]	Design	Temperature (°C)	Na_2CO_3 (g/L)	Oxygen (bar)	Reaction Time (minutes)
I1	d	185	2	6	15
I2	a	195	2	6	10
I3	b	185	6.5	6	10
I4	abd	195	6.5	6	15
I5	c	185	2	12	10
I6	acd	195	2	12	15
I7	bcd	185	6.5	12	15
I8	abc	195	6.5	12	10

3.3.1 Characteristics of the solid fraction

The wet oxidation gave a large variation in the chemical composition (**Table 19**). The cellulose, hemicellulose, lignin, and non-cell wall material (NCWM) contents were related to their original content in the starting material, M/M_0 (**Table 20**). The solid fraction retained 85-95 % cellulose, 5-46 % hemicellulose, 28-68 % lignin and 26-45 % NCWM (Klinke *et al*., 1998). To obtain an efficient fractionation of cellulose and hemicellulose, the relative characteristic of the solid fraction should be a high cellulose content and a low hemicellulose content. Thus treatment I4, I6, I7 and I8 obtained the best fractionation of cellulose and hemicellulose.

Table 19. *Chemical composition (% dry matter) of untreated and treated wheat straw 1997. Conditions as described in* ***Table 18****. NCWM: Non-cell wall material.*

Treatment	Solids (g)	Cellulose (%w/w)	Hemicellulose (%w/w)	Lignin (%w/w)	NCWM (%w/w)	Ash (%w/w)
Straw	60	37.0	27.9	8.7	25.1	1.4
I1	36.3	58.6	14.7	9.0	16.2	1.5
I2	36.2	58.0	12.3	9.7	18.6	1.3
I3	30.7	61.8	13.8	8.4	13.9	2.1
I4	28.4	67.4	8.0	7.7	13.7	3.2
I5	38.1	53.5	20.0	8.0	17.1	1.4
I6	28.6	65.1	3.2	7.1	21.8	2.7
I7	30.5	67.5	10.4	4.8	14.7	2.7
I8	29.3	67.8	7.6	5.6	15.7	3.3

Table 20. *The relative characteristics (M/M_0) of the solid fraction of wet-oxidised wheat straw 1997. Conditions as described in* ***Table 18****. M: Mass of component in solid fraction. M_0: Mass of component in starting material. NCWM: Non-cell wall material. (Klinke et al., 1998).*

Treatment	Cellulose (%)	Hemicellulose (%)	Lignin (%)	NCWM (%)
I1	95.9	31.9	63.0	38.9
I2	94.7	26.7	67.5	44.7
I3	85.6	25.3	49.7	28.3
I4	86.3	13.5	42.0	25.9
I5	91.9	45.5	58.7	43.2
I6	84.0	5.5	39.2	41.4
I7	92.8	19.0	28.1	29.6
I8	89.6	13.3	31.6	30.4

Pre-treatment I2 and I8 (**Table 18**) represent wet oxidation experiments with high (I8) and low (I2) oxygen and carbonate content. These were chosen as the two experiments for further investigation of phenolic compounds (se below). The lignin solubilisation (or breakdown) calculated as the loss from the solid fraction in I2

(32.5%) and I8 (68.4%) could mainly be attributed to the carbonate and oxygen added (**Table 20**). Also the higher solubilisation of the hemicellulose I8 compared with I2 could be addressed to the higher addition of carbonate and oxygen.

In general, the wet-oxidation experiments with a high residual content of hemicellulose and/or lignin resulted in low conversion of cellulose to glucose (**Table 21**). The enzymatic conversion of the treated cellulose fraction from experiment I8 was much higher than from experiment I2. This indicated that the removal of hemicellulose and/or lignin was important for obtaining a solid fraction assessable for enzyme treatment as previously found (Schmidt and Thomsen, 1998). An increase carbonate concentration had previously been found to increase the enzymatic convertibility of cellulose (Bjerre *et al.*, 1996a).

Table 21. *Effect of wet-oxidation conditions on enzymatic convertibility of the cellulose (% w/w) in the solid fraction to glucose for wheat straw 1997. Conditions as described in* ***Table 18****.*

I1	I2	I3	I4	I5	I6	I7	I8
38.1	39.3	53.7	63.2	38.1	66.3	62.1	67.7

3.3.2 Characteristics of the liquid fraction

3.3.2.1 Solubilised hemicellulose

In general, the concentration of solubilised hemicellulose after wet oxidation of wheat straw from 1997 (**Table 22**) was lower compared with wet oxidation of wheat straw from 1993 but higher compared with wet oxidation of wheat straw from 1994 (this report). An ANOVA test of the fractional design (**Table 18**) showed no significant effect of temperature, time, oxygen, and sodium carbonate addition within the tested range. The hemicellulose concentration ranged from 8.5 to 9.3 g sugar/L after acid hydrolysis (except for I5).

Table 22. *Effect of wet oxidation conditions on solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) for wheat straw 1997. Conditions as described in* ***Table 18****.*

Treatment	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total sugars (g/L)
I1	2.41	5.20	0.94	8.54
I2	2.25	6.11	0.91	9.27
I3	2.14	5.23	1.22	8.58
I4	1.70	6.03	1.51	9.24
I5	2.55	3.79	0.80	7.14
I6	1.94	6.30	0.59	8.83
I7	1.72	5.75	1.39	8.86
I8	1.54	5.96	1.40	8.90

3.3.2.2 Identification of potential inhibitors

Apart from hydrolysed hemicellulose, the liquid fraction derived from wet oxidation contained degradation products from hydrolysis, oxidation, or thermal decomposition of the individual constituents in lignocellulosic material (**Figure 2**). Phenolic monomeric compounds deriving from lignin degradation, acetic acid from hydrolysis of acetylated sugars, furan derivatives from thermal sugar degradation, and low molecular weight carboxylic acids from oxidation of both lignin and sugar compounds are considered to be potential fermentation inhibitors (Clark and Mackie, 1984; Delgenes *et al*., 1996).

Four wet oxidation experiments (I4, I6, I7, I8) resulted in very high amount of total carboxylic acids (> 6 g/L) (**Table 23**), which was much higher than previously found for straws at similar wet oxidation conditions (Schmidt and Thomsen, 1998; this report). Surprisingly, formic acid was the dominant acid and varied most with the treatment conditions, in contrast to that of wheat straw 1993 and 1994, where acetic acid was the dominant acid.

Table 23. *Effect of wet oxidation conditions on the formation of carboxylic acids from wheat straw 1997. Conditions as described in* ***Table 18****. (Klinke et al., 1998).*

Treatment	Formic (g/L)	Acetic (g/L)	Glycolic (g/L)	Malic (g/L)	Succinic (g/L)	Oxalic (g/L)	Total (g/L)
I1	0.4	0.9	0.4	0.1	0.1	0.004	1.9
I2	1.8	1.0	0.4	0.1	0.2	0.002	3.5
I3	1.3	1.2	0.3	0.1	0.5	0.016	3.3
I4	3.7	1.6	1.2	0.1	0.3	0.007	6.9
I5	2.2	1.0	0.4	0.1	0.2	0.005	3.8
I6	3.9	1.4	0.7	0.1	0.3	0.008	6.3
I7	4.3	1.5	0.9	0.2	0.2	0.007	7.2
I8	3.5	1.3	0.9	0.2	0.2	0.006	6.0

GC-MS is a well-established technique to identify and quantify compounds from complex mixtures also in very low concentrations. Trimethylsilylation (TMS) of less volatile compounds with hydroxylic groups makes it possible to screen the liquid fractions for a vast number of low molecular weight compounds such as monomeric phenols and carboxylic acids, both aromatics and aliphatics (Niemelä and Sjöström, 1986). Wet oxidation experiments I2 and I8 were selected for in dept analysis by GC-MS due to very different treatment conditions (Klinke *et al*., 1998). The monomeric phenolic products could be divided into three main structures consisting of a para-substituted phenol with zero, one or two methoxy groups ortho to the phenol hydroxyl group.

All three basic structures were identified as their phenol aldehydes, ketones or acetophenones and acids (**Table 24**). The total content of phenolic compounds was about the same in the two experiments, but the total content of fatty acids was much higher in experiment I8 than in experiment I2. In general, the amount of carboxylic acids (**Table 23**) was about a factor 50 higher than the amount of phenolic compounds (**Table 24**). In wet oxidation experiment I2, the solubilisation of hemicellulose and lignin from the solid fraction was lower than that of experiment I8 (**Table 20**). As the content of solubilised hemicellulose was higher in experiment I2 than in experiment I8 (**Table 22**) this indicated that more hemicellulose was converted to other products in experiment I8 *e.g.* to carboxylic acids. Besides, more lignin was removed from the solid fraction in experiment I8 indicating that more phenolic compounds were converted into carboxylic acids. The conversion of phenols and hemicellulose into low molecular weight carboxylic acids might also be explained by the harsher oxidising conditions of experiment I8 (12 bar O_2) compared to those of experiment I2 (6 bar O_2).

Table 24. *Semiquantitative determination by GC-MS of phenolics in experiments I2 and I8. Sample preparation: SPE extraction at pH 7 (a1) or pH 2 (a2) and (b) freeze-dried and silylated. Wet oxidation conditions as described in* ***Table 18****. (Klinke et al., 1998).*

Compounds identified	Structure (see **Figure 2**)			Sample prep.	Conc. I2	Conc. I8
	R_1	R_2	R_3	-	(mg/L)	(mg/L)
phenol	H	H	H	a1	1	2
guaiacol	H	H	OCH_3	a1	3	6
syringol	H	OCH_3	OCH_3	a1	1	1
4-hydroxybenzaldehyde	CHO	H	H	a1	6	6
vanillin	CHO	H	OCH_3	a1	32	24
syringaldehyde	CHO	OCH_3	OCH_3	a1	19	12
3,4,5-trimethoxybenzaldehyde*	CHO	OCH_3	OCH_3	a1	<1	<1
4-hydroxy acetophenone	$COCH_3$	H	H	a2	<1	2
acetovanillone	$COCH_3$	H	OCH_3	a2	2	4
4-hydroxy-3,5-dimethoxyacetophenone	$COCH_3$	OCH_3	OCH_3	a2	15	18
3,4,5-trimethoxyacetophenone*	$COCH_3$	OCH_3	OCH_3	a2	<1	<1
2-furoic acid	-	-	-	a1	4	7
4-hydroxybenzoic acid	COOH	H	H	a1	3	9
vanillic acid	COOH	H	OCH_3	a1	17	68
syringic acid	COOH	OCH_3	OCH_3	a1	17	22
coumaric acid	CH=CHCOOH	H	H	a1	15	11
ferulic acid	CH=CHCOOH	H	OCH_3	a1	10	10
4-hydroxy-3-methoxy-phenethylene glycol	$CHOHCH_2OH$	H	OCH_3	b	<1	<1
Total concentration	-	-	-	-	145	202

*) Phenol hydroxyl group was methylated

The production of furfurals varied in the eight different wet oxidation experiments depending of the final pH-value measured (**Table 25**). As seen before, higher concentrations of 2-furfural were found at low pH (up to 80 mg/L) and no or only small amounts (1-2 mg/L) were found at high pH as indicated in **Figure 8** (Schmidt and Thomsen, 1999). The same tendency was found for 5-hydroxymethyl-2-furfural but at approximately 10 times lower concentration than for 2-furfural. In general, high pH-value gave a higher level of total organic carbon.

Table 25. *Effect of wet oxidation conditions on the formation of furfurals and total organic carbon (TOC) from wheat straw 1997. Conditions as described in* ***Table 18****.*

Compounds (mg/L)	I1	I2	I3	I4	I5	I6	I7	I8
pH	4.8	4.7	7.7	6.1	4.7	3.8	6.0	5.9
2-Furfural	13.3	19.1	0	0	1.7	80.6	0.4	0.7
5-Hydroxymethyl-2-furfural	0.8	0.5	0	0	0	9.2	0	0
TOC (g C/L)	13.6	13.6	16.5	18.7	12.5	16.1	17.5	18.2

3.3.2.3 Mass balances - polysaccharides

The recoveries for wheat straw 1997 (**Table 26**) were similar to those of wheat straw 1993 and 1994. The recoveries for cellulose were very high whereas the ones for hemicellulose were low as seen for wheat straw from 1993 (60%) and 1994 (50%) (Schmidt and Thomsen, 1998). In general, wet oxidation conditions I7 and I8 were found to be the best for fractionation of wheat straw 1997 based on hemicellulose solubilisation, cellulose convertibility and recovery. In a detailed mass balance on the liquid fraction from alkaline wet oxidation, the solubilised hemicellulose accounted for 33% of the measured total TOC (mg C/L), low molecular weight carboxylic acids for approximately 20%, furfurals for 0.02% and phenolic compound for 1.5% (data not shown) (Klinke *et al*., 1999). The rest remained unidentified but is presumably high molecular weight components.

Table 26. *Effect of wet oxidation conditions on the calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) for wheat straw 1997. Conditions as described in* ***Table 18****.*

Treatment	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
I1	64.1	105.6	87.8
I2	63.5	103.7	86.4
I3	59.2	94.1	79.1
I4	53.2	93.1	76.0
I5	69.6	102.2	88.2
I6	41.7	91.7	70.2
I7	56.5	99.7	81.1
I8	52.0	95.7	76.9

3.4 Birchwood

The investigation of wet oxidation for fractionation of wood was based on the optimal wet oxidation conditions found for wheat straw 1993: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, and 15 minutes. In this study, mainly birchwood was used for comparison purposes. Birchwood is a typical hardwood where the main building block in hemicellulose is xylose just like in wheat straw. However, the acetyl content is significantly higher. In birchwood xylan, approximately every tenth xylose unit carries a 4-O-methyl-glucuronosyl substituent. In addition 7 out of ten xylose units are acetylated (Bouveng, 1961). The acetyl groups are linked either to C2 or C3 position or to both in the ratio 24:22:10 (Lindberg *et al*., 1973).

3.4.1 Effect of temperature

As the temperature was found to be the most important wet oxidation parameter (Schmidt and Thomsen, 1998), the effect of temperature on the degree of fractionation and cellulose convertibility was investigated at two different reaction times (**Figure 16**). The two different reaction times gave very different results. At 10 minutes reaction time, the best convertibility seemed to be obtained at a temperature around 185°C (**Figure 16a**). Whereas at 15 minutes reaction time, the best convertibility was obtained at a much higher temperature (200°C) (**Figure 16b**). This was very surprising as normally a reaction at lower temperature was expected to run for longer than at higher temperature to obtain an efficient conversion. The value of the cellulose convertibility was similar for both reaction times.

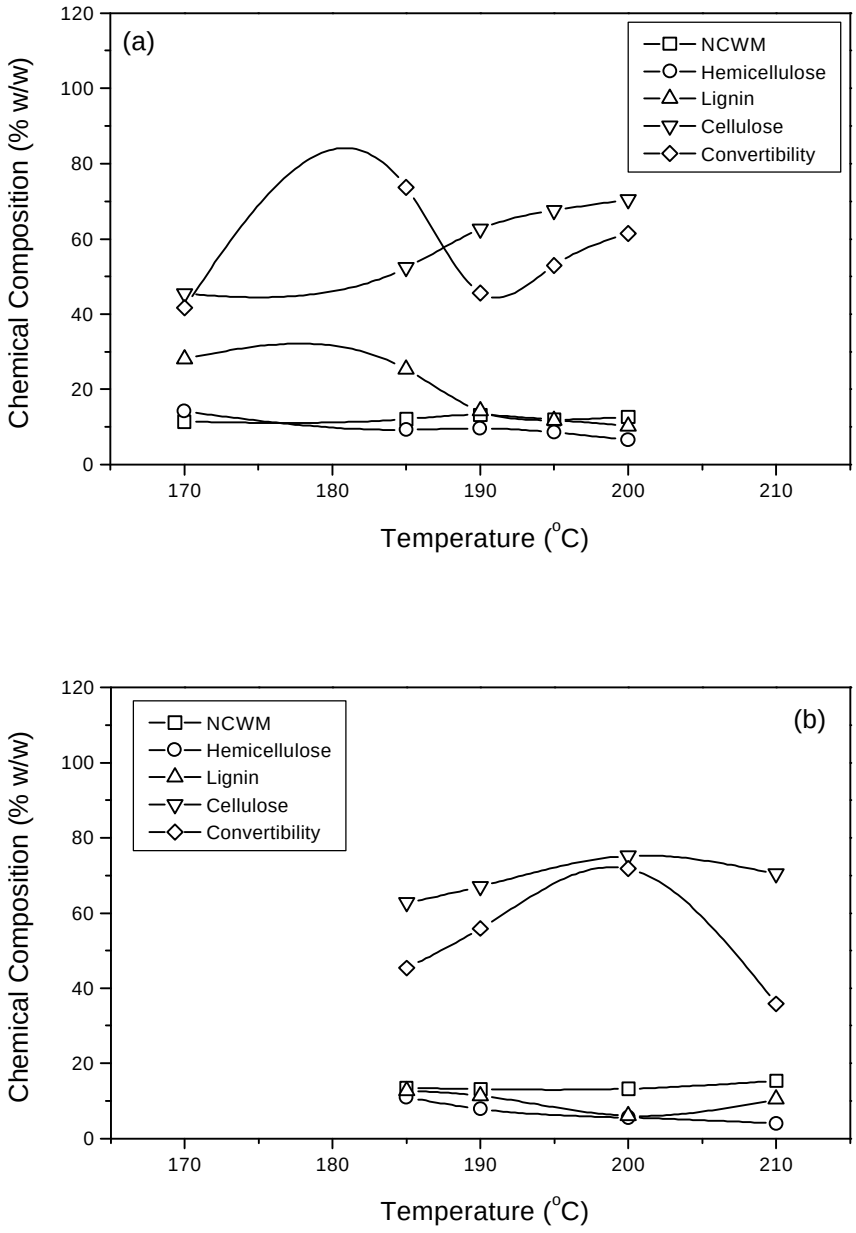

Figure 16. *Effect of wet oxidation temperature on the chemical composition (% dry weight) (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) and the cellulose convertibility of the solid fibre fraction from birchwood at two different reaction times (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2).* ***a)*** *10 minutes.* ***b)*** *15 minutes.*

The two different reaction times also remove the different components very differently (**Figure 17**). At 10 minutes reaction time, the lignin component only began to be removed at temperatures above 185°C (**Figure 17a**). At low temperatures only 80% of the cellulose remained as a solid, whereas this increased at higher temperatures (to 95%), where 50-60% of the lignin was removed. At 15 minutes reaction time, about 90% of the cellulose remained as a solid at all temperatures, whereas much more lignin (60-80%) was removed (**Figure 17b**) than at 10 minutes reaction time. The hemicellulose removal (80-90%) increased slightly with temperature at both reaction times. It was noted that only 40-50% of the non-

cell wall material was removed from the solid fraction in contrast to 60-80% for wheat straw. However, the effect of temperature was similar for both wet oxidation times. A temperature of 200°C maintained most cellulose in the solid fraction and removed most lignin.

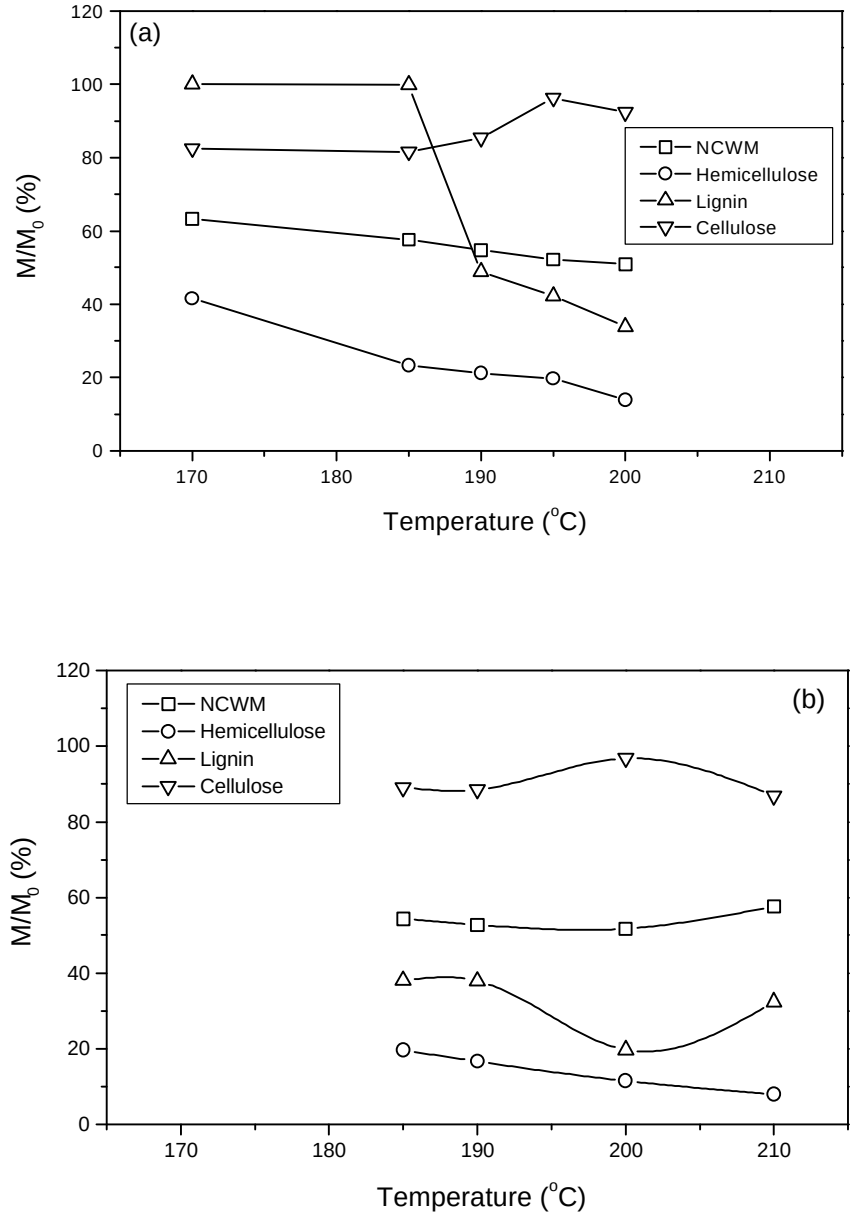

Figure 17. *Effect of wet oxidation temperature on the relative characteristics (M/M_0) of non-cell wall material (NCWM), hemicellulose, lignin, and cellulose from the solid fibre fraction of birchwood at two different reaction times (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2).* ***a)*** *10 minutes.* ***b)*** *15 minutes. M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

The best temperature for solubilisation of hemicellulose (measured as monosaccharides after acid hydrolysis) was different at the two reaction times though the overall amount was very similar (**Figure 18**). Surprisingly, a longer reaction time (15 minutes) required a higher temperature (200°C) than a shorter time of 10 minutes (185-195°C). Only about 4 g/L of hemicellulose was solubilised in

both cases, which was very low compared to about 10 g/L for wheat straw at similar wet oxidation conditions. Xylose was nearly the only sugar formed after acid hydrolysis of the hemicellulose fraction. Only traces of arabinose and glucose could be determined and xylose accounted for about 95% of the measured solubilised hemicellulose.

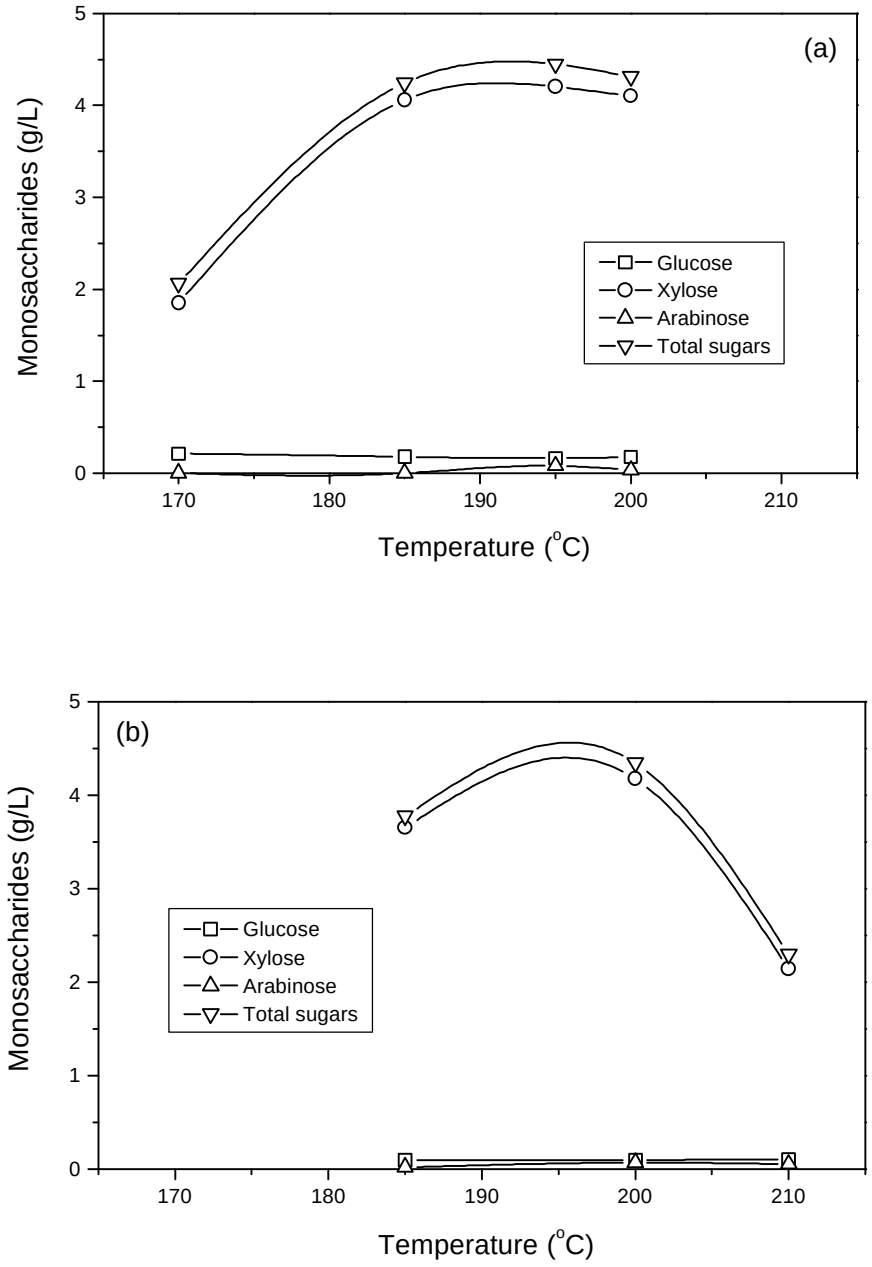

Figure 18. *Effect of wet oxidation temperature on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from birchwood at two different reaction times (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2).* ***a)*** *10 minutes.* ***b)*** *15 minutes.*

The amount of formed carboxylic acids varied with the reaction temperature at both reaction times (**Figure 19**). For a reaction time of 10 minutes, the highest acid concentration was obtained at 195°C, whereas 200°C gave the highest acid concentration for a reaction time of 15 minutes. Acetic acid was the most dominant

acid probably due to the high acetyl content in birchwood hemicellulose. The released acetic acid was at the same concentration level as the released hemicellulose sugars. Surprisingly, significantly more carboxylic acids (up to 8.3 g/L) than solubilised hemicellulose (4.5 g/L) (**Figure 18b**) were found at 200°C after 15 minutes reaction time.

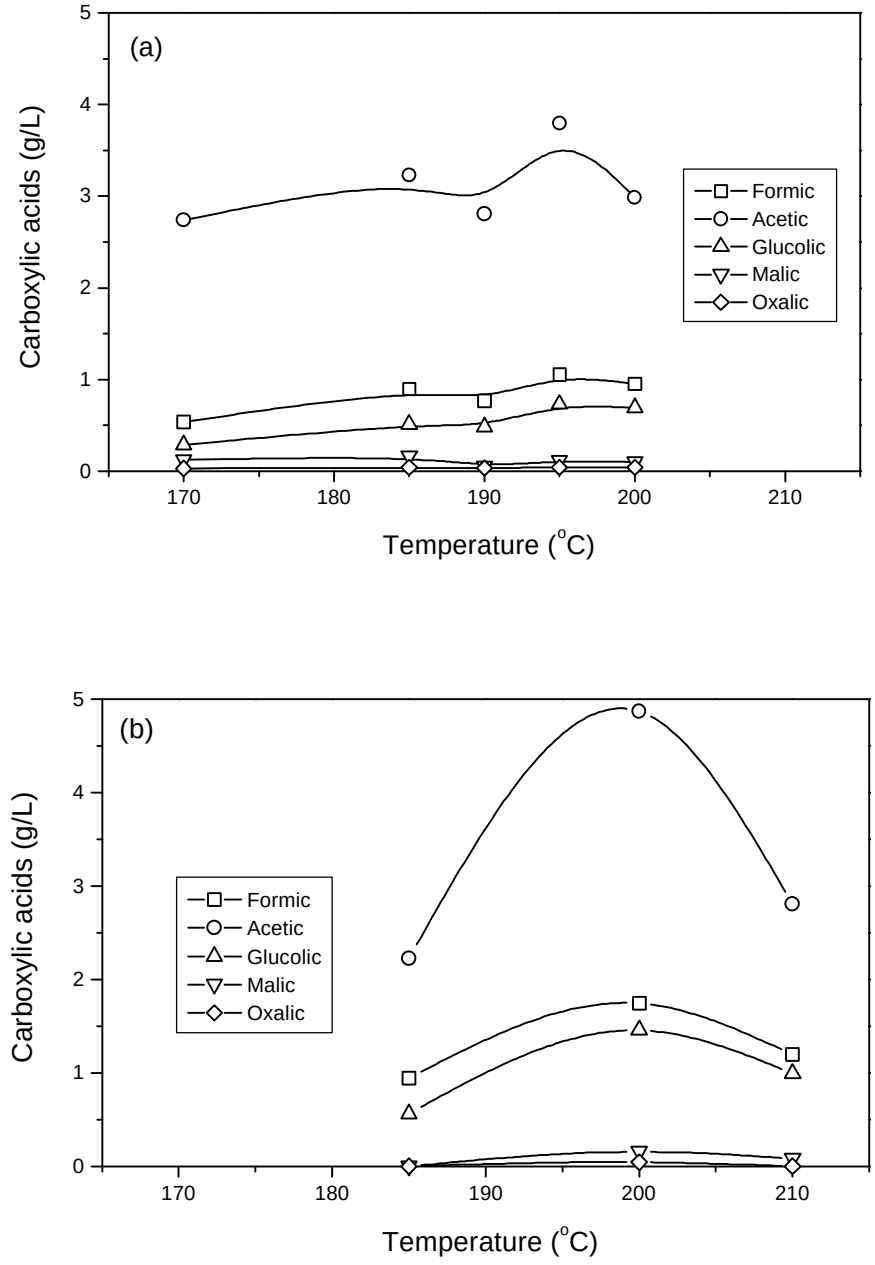

Figure 19. *Effect of wet oxidation temperature on formation of carboxylic acids from birchwood at two different reaction times (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2).* ***a)*** *10 minutes.* ***b)*** *15 minutes.*

The formation of the sugar degradation products, 2-furfural and 5-hydroxymethyl-2-furfural, increased with temperature (**Table 27**) as previously found for wheat straw. The highest level of total organic carbon was found at 200°C, which also gave the most hemicellulose and carboxylic acids in solution.

Table 27. *Effect temperature on formation of 2-furfural, 5-hydroxymethyl-2-furfural (HMF) and total organic carbon (TOC) from wet oxidation of birchwood (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Compound	185°C	190°C	200°C	210°C
pH	5.8	5.3	4.7	4.6
2-Furfural (mg/L)	0	1.5	20.7	49.3
HMF (mg/L)	0.5	1.3	2.3	0.6
TOC (g C/L)	7.2	8.1	9.2	7.7

3.4.2 Effect of reaction time

As the reaction time previously was found to have a large effect on the fractionation (Schmidt and Thomsen, 1998), this wet oxidation parameter was also investigated for birchwood. The chemical composition of the solid fibre fraction did not vary much with reaction times longer than 5 minutes giving a composition of about 70% cellulose, and 10% of non-cell wall material, hemicellulose, and lignin (**Figure 20**). However, the cellulose convertibility significantly increased with wet oxidation time, from 35% after 5 minutes to about 60% at 25 minutes.

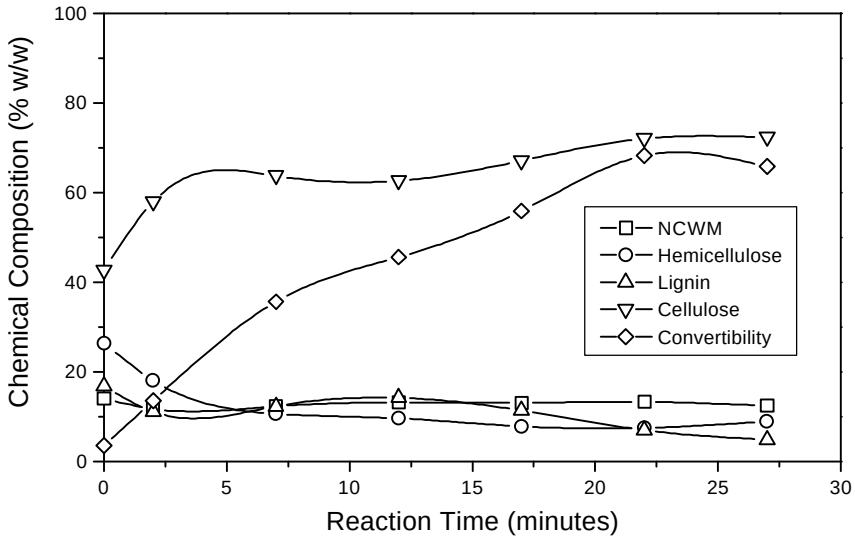

Figure 20. *Effect of wet oxidation time on the chemical composition (% dry weight) (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) and cellulose convertibility of the solid fibre fraction of birchwood (60 g/L wood, 190°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

The degree of solubilisation did not vary much at wet oxidation times longer than 5 minutes (**Figure 21**). Surprisingly, 15% of the cellulose had been removed from the solid fraction, which was much higher than the maximal 5% removed by wet oxidation of wheat straw (Schmidt and Thomsen, 1998, this report). About 50% of the non-cell wall material were removed. At reaction times longer than 10 minutes, more than 90% of the hemicellulose and lignin was removed from the solid fibre fraction. The 90% original hemicellulose removed from the solid fraction would correspond to about 14.5 g/L hemicellulose in the liquid fraction at 100% recovery. However, a very low concentration of hemicellulose was found in the liquid fraction (**Figure 22**) reaching 4 g/L after 25 minutes (only about 30% of the solubilised hemicellulose).

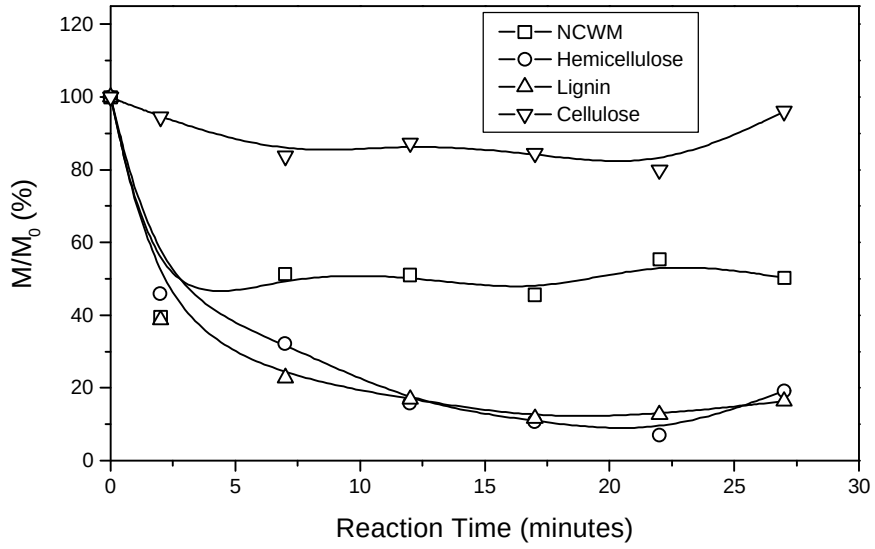

Figure 21. *Effect of wet oxidation time on the relative characteristics (M/M_0) of non-cell wall material (NCWM), hemicellulose, lignin, and cellulose from the solid fibre fraction of birchwood (60 g/L wood, 190°C, 6.5 g/L Na_2CO_3, 12 bar O_2). M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

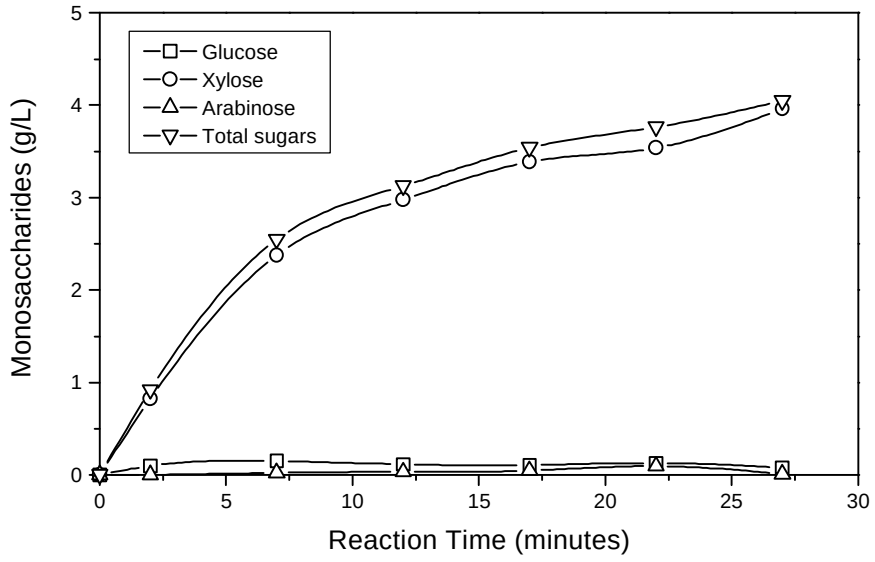

Figure 22. *Effect of wet oxidation time on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from birchwood (60 g/L wood, 190°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

The final pH-value decreased with the wet oxidation time, which probably led to the increasing level of furfurals (**Table 28**). Furthermore, the total organic carbon (TOC) solubilised in the liquid fraction also increased with the wet oxidation time.

Table 28. *Effect of reaction time on formation of 2-furfural, 5-hydroxymethyl-2-furfural (HMF) and total organic carbon (TOC) from wet oxidation of birchwood (60 g/L wood, 190°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

Reaction time (min)[a]	2	7	12	17	22	27
pH	8.0	7.9	7.1	5.3	5.0	4.7
2-Furfural (mg/L)	0	0	0	1.5	5.8	16.1
HMF (mg/L)	0	0	0.6	0.7	0	2.5
TOC (g C/L)	2.8	5.2	6.2	8.1	8.7	-[b]

a: Reaction times are included the 2 minutes heating and cooling times.
b: Analysis not performed.

The recovery was calculated for the two polysaccharides, hemicellulose and cellulose, based on mass balances (**Table 29**). There was no clear pattern as regard to the effect of the wet oxidation time. The recoveries for hemicellulose were very low (30-40%) compared to wheat straw (50-70%) (Schmidt and Thomsen, 1998; this report). The recoveries for cellulose were similar to that of wheat straw. But due to the very low hemicellulose recovery, the overall recoveries were low (about 75%).

Table 29. *Effect of reaction time on the calculated recovery (%) based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) obtained from wet oxidation of birchwood (60 g/L wood, 190°C, 6.5 g/L Na_2CO_3, 12 bar O_2).*

Reaction time (min)[a]	2	7	12	17	22	27
Hemicellulose	28.1	41.2	45.7	42.8	36.7	41.7
Cellulose	115.6	103.6	92.8	89.4	96.0	96.2
Total polysaccharides	82.2	79.7	74.8	71.6	73.3	75.4

a: Reaction times are included the 2 minutes heating and cooling times.

3.4.3 Effect of oxygen and alkaline

In general, alkaline wet oxidation of birchwood gave poor hemicellulose recovery and in particular low concentration of solubilised hemicellulose compared to that of wheat straw. For birchwood, significantly higher hemicellulose solubilisation had been achieved by steaming (Schmidt *et al*., 1996). Hence, wet oxidation was compared to hydrothermal treatment in the absence of oxygen pressure and/or alkaline at 190°C and 200°C. For both temperatures, the highest convertibility and concentration of cellulose in the solid fibre fraction was achieved by alkaline wet oxidation (**Figure 23**). The higher temperature (200°C) resulted in 70% conversion of cellulose (75% w/w cellulose of the fibre) to glucose probably due to the low lignin content. This conversion was comparable to that of wet oxidised wheat straw. For 190°C, only 55% conversion could be obtained, which was far too low for further analysis, hence, only the experiments at 200°C were analysed further. The high convertibility at 200°C was probably obtained as more than 80% of the original lignin had been removed from the solid fraction (**Figure 24**). In the two experiments where oxygen was absent, much more lignin remained (60-70%) in the solid fraction. The best fractionation of birchwood was obtained during the wet oxidation without alkaline at 200°C where all hemicellulose was solubilised, however, more than 25% of the cellulose was also removed from the solids.

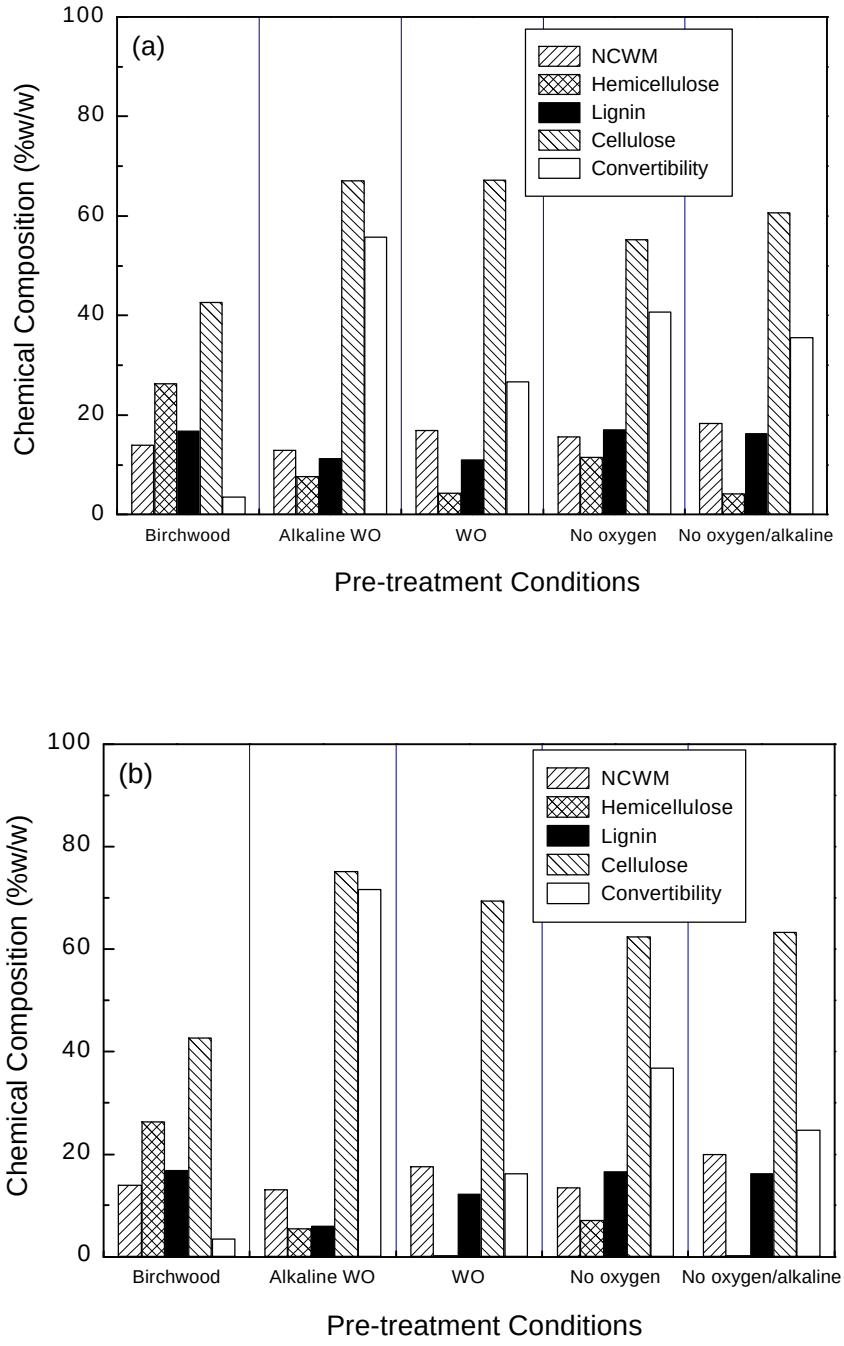

Figure 23. *Chemical composition (non-cell wall material (NCWM), hemicellulose, lignin and cellulose) (%w/w dry weight) and the enzymatic cellulose convertibility to glucose of the solid fraction after pre-treatment of birchwood at two different temperatures (Alkaline WO: 60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes). WO: Wet oxidation.* ***a)*** *190°C.* ***b)*** *200°C.*

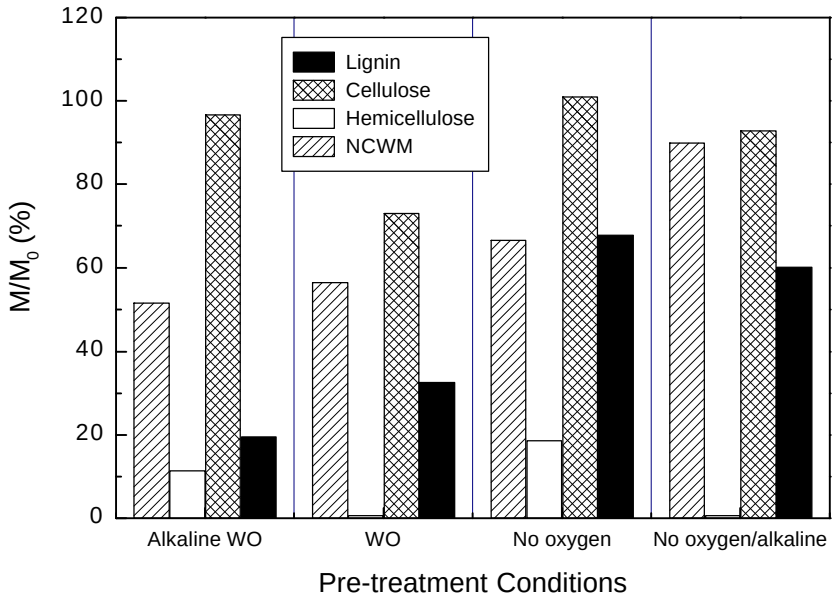

Figure 24. *Relative characteristics (M/M_0) of non-cell wall material (NCWM), hemicellulose, lignin and cellulose in the solid fraction obtained by pre-treatment of birchwood (Alkaline WO: 60 g/L wood, 200°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material. WO: Wet oxidation.*

For birchwood, the addition of oxygen and alkaline in the pre-treatment process had a significant effect on the amount of solubilised hemicellulose (**Figure 25**). By using alkaline wet oxidation very low concentration of solubilised hemicellulose (< 4 g/L) was obtained. By removing the alkaline, significantly higher hemicellulose concentration could be found in the liquid fraction (8-10 g/L). Surprisingly, the highest amount of solubilised hemicellulose was achieved in the absent of both chemicals at 200°C. Hence, in order to obtain hemicellulose for fermentation, pre-treatment should be performed without alkaline. However, this then resulted in low convertibility of the cellulose in the solid fraction making it difficult to use both polysaccharide fractions for fermentation.

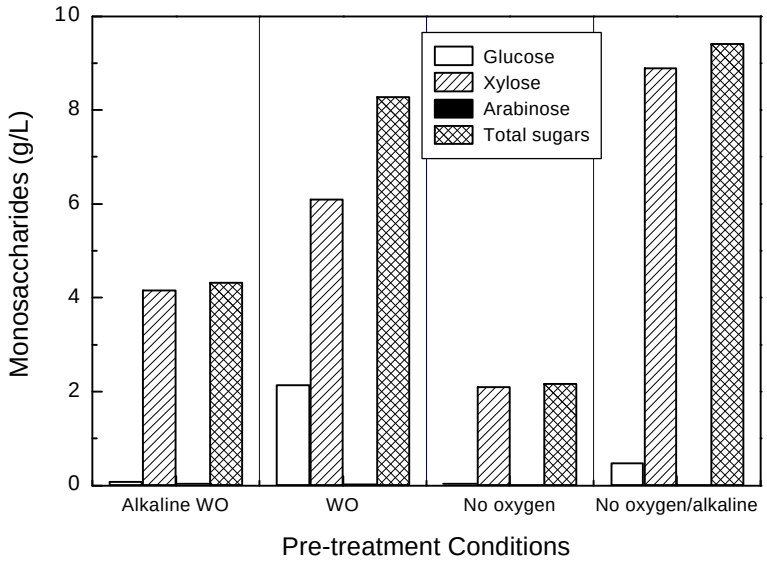

Figure 25. *Solubilisation of hemicellulose (measured as monosaccharides after acid hydrolysis) obtained by pre-treatment of birchwood (Alkaline WO: 60 g/L wood, 200°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes). WO: Wet oxidation.*

Several carboxylic acids were formed during pre-treatment of birchwood (**Table 30**), where acetic acid was the dominant acid, due to the high acetyl content in birchwood. Both oxygen and alkaline seemed to have an effect on the formation. For wet oxidation with or without alkaline 60% of total acids was acetic acid, when oxygen was absent in the process up to 80% of the total acids was acetic acid. When both chemicals were absent only low formation of acids occurred (1.8 g/L). During alkaline wet oxidation, the highest concentration of acids was obtained (8.3 g/L) which was a much higher than the concentration of solubilised hemicellulose (around 4 g/L). To avoid formation of carboxylic acids (potential inhibitors during the succeeding fermentation) either oxygen or alkaline should be absent from the reaction mixture. However, if alkaline was not added to the reaction mixture high formation of furfurals occurred (**Table 31**), compared to that of wheat straw (Schmidt and Thomsen, 1999).

Table 30. *Formation of carboxylic acids (g/L) by pre-treatment of birchwood (Alkaline WO: 60 g/L wood, 200°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes). WO: Wet oxidation.*

Carboxylic acids	Alkaline WO	WO	No oxygen	No oxygen/alkaline
Formic	1.7	1.6	0.36	0
Acetic	4.9	3.4	3.6	1.4
Glycolic	1.5	0.52	0.23	0.12
Malic	0.16	0.14	0.09	0.10
Oxalic	0.09	0.05	0.01	0.01
Succinic	0.04	0	0	0
Citric	0	0.06	0.05	0
Iso-butyric	0	0	0	0.12
Total acids	8.3	5.8	4.3	1.8

Table 31. *Formation of furfurals (2-furfural and 5-hydroxymethyl-2-furfural (5-HMF)) and the total organic carbon (TOC) from pre-treatment of birchwood (Alkaline wet oxidation: 60 g/L wood, 200°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes).*

Pre-treatment	pH	2-Furfural (mg/L)	5-HMF (mg/L)	TOC (g C/L)
Alkaline wet oxidation	4.6	27	2.1	9.2
Wet oxidation	2.4	391	20	10.3
No oxygen	7.5	0	0	4.9
No oxygen/alkaline	3.5	402	43	7.7

The addition of oxygen pressure and alkaline during pre-treatment of birchwood resulted in low recovery for hemicellulose (35%) and high for cellulose (95-100%) (**Table 32**). The only pre-treatment conditions that gave a reasonable recovery for hemicellulose (50%) were when both oxygen and alkaline were absent. When alkaline was removed from the wet-oxidation process, lower recovery for cellulose was obtained (80%). The conditions giving the best overall recovery of the polysaccharides were when both oxygen and alkaline were absent from the process. These conditions also gave the highest concentration of solubilised hemicellulose **(Figure 25**). However, the convertibility of the cellulose was very low (<30%). Therefore, to find optimal conditions for cellulose convertibility, hemicellulose solubilisation and polysaccharide recovery can be tedious. More detailed experiments is needed to fully understand the mechanism governing the fractionation process.

Table 32. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) after pre-treatment of birchwood (Alkaline wet oxidation: 60 g/L wood, 200°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes).*

Pre-treatment	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
Alkaline wet oxidation	34.4	97.1	73.1
Wet oxidation	35.0	79.5	62.5
No oxygen	30.6	101.2	74.2
No oxygen/alkaline	50.3	94.4	77.5

3.5 Willow

Willow, a purpose grown crop, was investigated for using wet oxidation to make the polysaccharides available for conversion purposes. Similar wet oxidation conditions were applied in order to compare results from willow with those of wheat straw and birchwood. However, only a few experiments were performed. Willow is, like birchwood, a hardwood rich in xylose.

3.5.1 Effect of temperature

The effect of alkaline wet oxidation temperature on the chemical composition of the solid fibre fraction was small (**Figure 26**), however, the hemicellulose content decreased with temperature (from 20% to 5%). By increasing the temperature (to 200°C) an increase in cellulose convertibility was observed (up to 50%). This convertibility was still considerably lower than what was obtained for wheat straw (Schmidt and Thomsen, 1998). During alkaline wet oxidation nearly all of the original cellulose remained in the solid fraction (**Figure 27**), whereas more and more lignin and hemicellulose were removed from the solid fibre fraction as temperature

increased. As for birchwood, there was no effect of the temperature on the removal of non-cell wall material (75% of the original was removed).

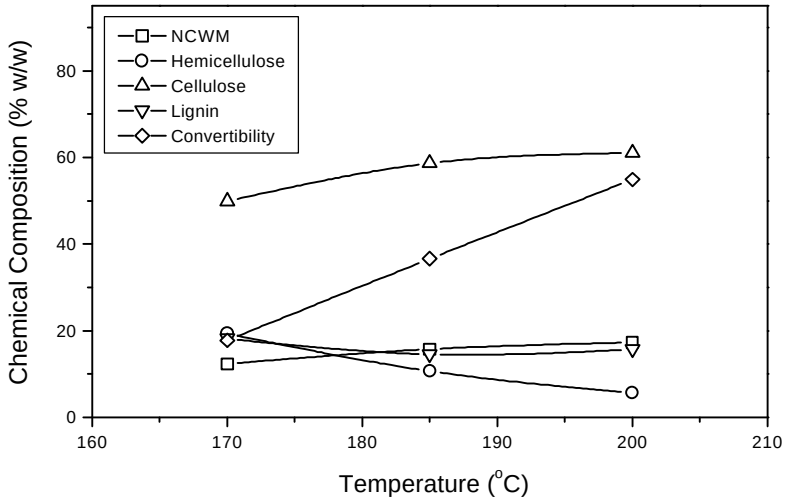

Figure 26. *Effect of wet oxidation temperature on the chemical composition (% dry weight) (non-cell wall material (NCWM), hemicellulose, lignin, and cellulose) and cellulose convertibility of the solid fraction from willow (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

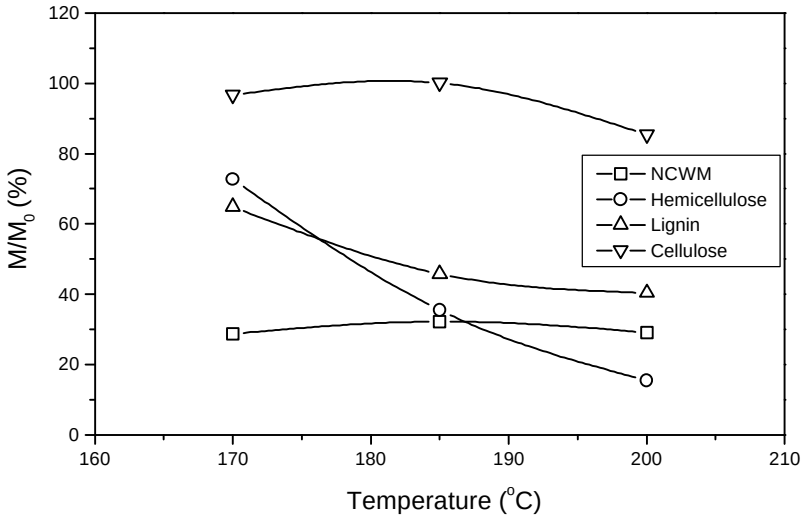

Figure 27*. Effect of wet oxidation temperature on the relative characteristics (M/M_0) of non-cell wall material (NCWM), hemicellulose, lignin and cellulose in the solid fraction of willow (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

The amount of solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) had the same temperature optimum (185°C) (**Figure 28**) as wheat straw 1993 (Schmidt and Thomsen, 1998). However, the concentration of hemicellulose was much lower (ca. 5.5 g/L) for willow than for wheat straw (ca. 10 g/L). The temperature effect was mainly observed in the amount of measured xylose and hardly any effect was seen on the glucose and arabinose concentration. The formation of furfurals was strongly dependent on the final pH-value after wet

oxidation (**Figure 29**). As the pH-value decreased with temperature, the formation of furfurals increased. The concentration of furfurals was higher than that of wheat straw but lower than that of birchwood.

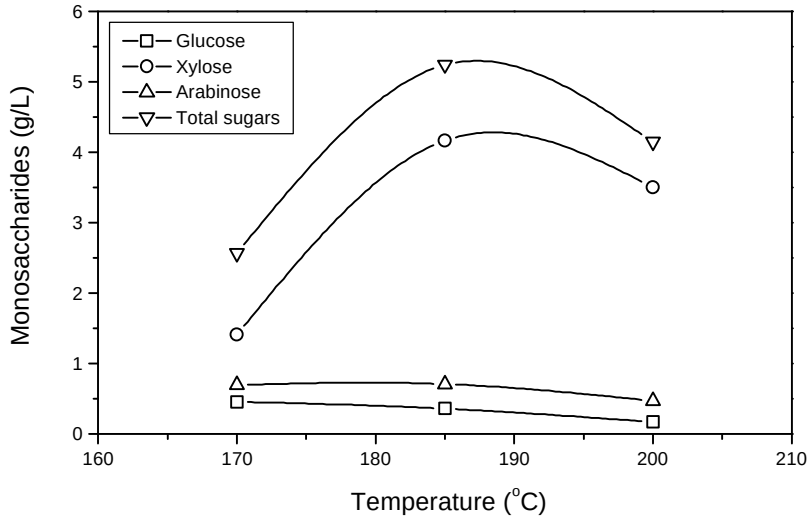

Figure 28. *Effect of wet oxidation temperature on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from willow (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

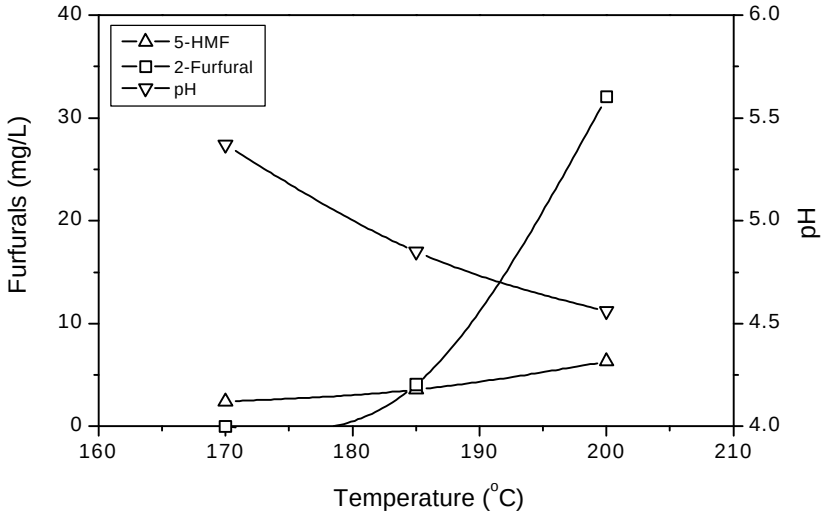

Figure 29. *Effect of wet oxidation temperature on formation of furfurals (2-furfural and 5-hydroxymethyl-2-furfural (5-HMF)) and final pH-value from willow (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

The recoveries of the two polysaccharides, hemicellulose and cellulose, were very good (near 100%) except for hemicellulose at 200°C (**Table 33**). At the two lower temperatures (170°C and 185°C) all polysaccharides were recovered. It is encouraging that such high recoveries for hemicellulose can be obtained by wet oxidation.

Table 33. *Effect of wet oxidation temperature on the calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) from willow (60 g/L wood, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Temperature (°C)	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
170	102.0	109.0	106.6
185	89.5	112.3	104.6
200	58.1	95.0	82.5

3.5.2 Effect of oxygen and alkaline

As previously described for wheat straw (Schmidt and Thomsen, 1999) and birchwood (this report), the addition of oxygen and alkaline in the pre-treatment process influenced the fractionation. For willow, the wet oxidation with and in particular without alkaline addition gave a good fractionation, where the cellulose content was high and the hemicellulose content low (**Figure 30**). The wet oxidation without alkaline addition also gave the highest convertible cellulose (about 50%). This was much lower than that of wheat straw and birchwood.

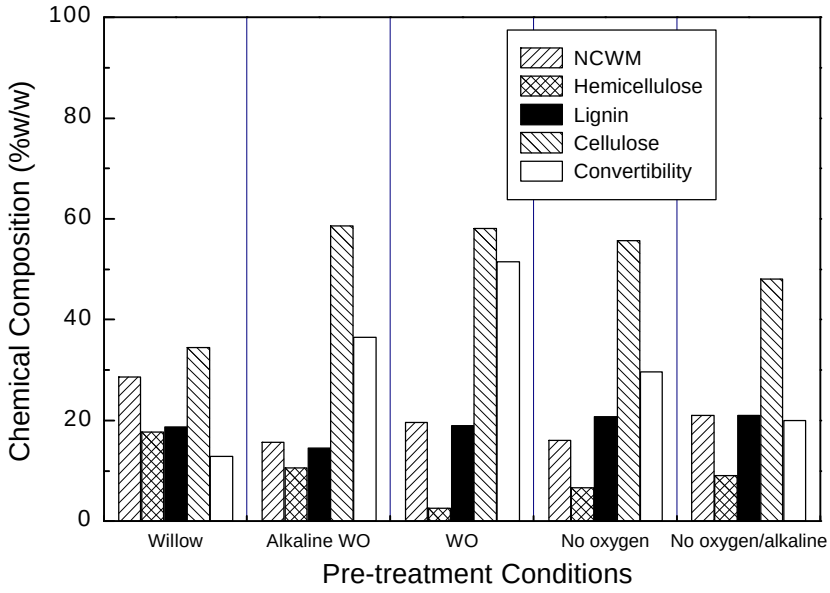

Figure 30. *Chemical composition (non-cell wall material (NCWM), hemicellulose, lignin and cellulose) (%w/w dry weight) and the enzymatic cellulose convertibility to glucose of the solid fraction after hydrothermal treatment of willow (Alkaline WO: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes). WO = Wet oxidation.*

Most lignin was removed during the alkaline wet oxidation (60%) (**Figure 31**) which should give the highest convertibility. However, wet oxidation without alkaline gave significantly the highest convertibility of 53%. In this experiment, only 50% of the lignin was removed but almost all the hemicellulose had been removed (> 90%). Treatment with water alone (no oxygen/alkaline) only removed about 20% of the original lignin resulting in the very low observed convertibility (**Figure 30**). All the different treatments hardly removed any cellulose from the solid fraction (<10%).

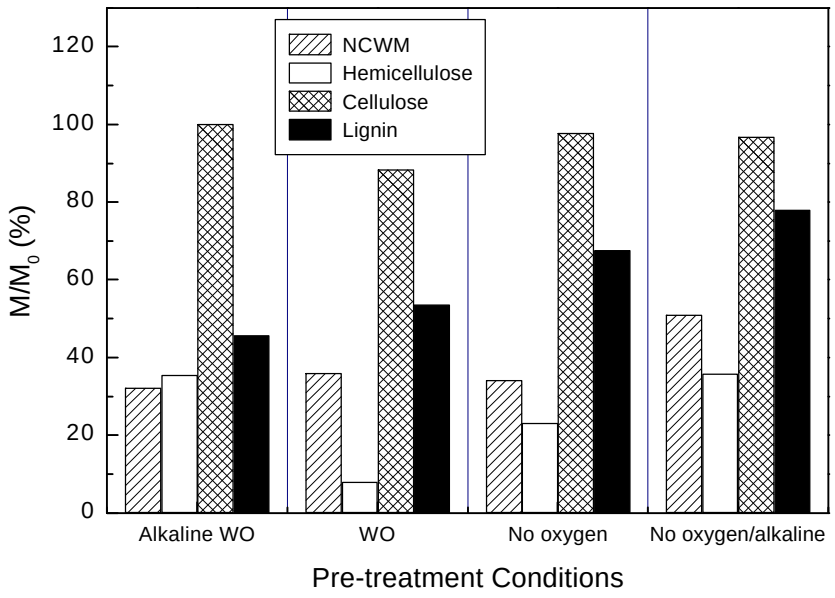

Figure 31. *Relative characteristics (M/M_0) of non-cell wall material (NCWM), hemicellulose, lignin and cellulose in the solid fraction obtained by hydrothermal treatment of willow (Alkaline WO: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material. WO = Wet oxidation.*

As previously described for wheat straw and birchwood, the addition of oxygen and alkaline in the pre-treatment process influenced the solubilisation of hemicellulose. For willow, the effect of the two chemicals was significant, where the addition of alkaline resulted in a somewhat lower hemicellulose concentration in the liquid fraction (**Table 34**). The highest amount of solubilised hemicellulose was achieved for wet oxidation with alkaline absent in accordance with fractionation efficiency (**Figure 30**). Hence, these were the same conditions giving highest convertibility. For willow as for wheat straw, the absence of both chemicals resulted in the lowest solubilisation of hemicellulose.

Table 34. *Solubilisation of hemicellulose (measured as monosaccharides after acid hydrolysis) obtained by pre-treatment of willow (Alkaline wet oxidation: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2 and 15 minutes).*

Pre-treatment	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total sugar (g/L)
Alkaline wet oxidation	0.4	4.2	0.7	5.2
Wet oxidation	1.7	6.1	0.4	8.2
No oxygen	0.3	3.4	0.7	4.4
No oxygen/alkaline	0.6	1.9	0.3	2.7

The formation of furfurals was dependent on the pre-treatment conditions (**Table 35**). At low final pH-value, significantly more furfurals were formed, whereas when alkaline was added to the process only low or no furfurals were formed. Surprisingly, 2-furfural and 5-hydroxymethyl-2-furfural were formed in similar amount.

Table 35. *Formation of furfurals (2-furfural and 5-hydroxymethyl-2-furfural (5-HMF)) and final pH-value by pre-treatment of willow (Alkaline wet oxidation: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Pre-treatment	2-Furfural (mg/L)	5-HMF (mg/L)	pH
Alkaline wet oxidation	4.1	3.6	4.9
Wet oxidation	124	143	3.0
No oxygen	0	0	7.0
No oxygen/alkaline	80.2	160	3.7

The recovery of the two polysaccharides depended on the pre-treatment conditions (**Table 36**). In the absence of alkaline, the recovery of hemicellulose was lower (54-61%) than when alkaline was present (75-78%). All cellulose was recovered except when willow was treated by wet oxidation without alkaline. These were the conditions, where the highest cellulose convertibility and highest amount of solubilised hemicellulose were achieved, but the overall recovery was only 84%. However, in general the recoveries for willow were quite high.

Table 36. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) after pre-treatment of willow (Alkaline wet oxidation: 60 g/L wood, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Pre-treatment	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
Alkaline wet oxidation	75.2	101.7	92.7
Wet oxidation	61.4	95.9	84.1
No oxygen	77.5	99.3	91.9
No oxygen/alkaline	53.8	99.2	83.8

3.6 Sugar beet pulp

Sugar beet pulp, an agroindustrial waste product, was investigated using wet oxidation for making the polysaccharides available for fermentation purposes. Several different wet oxidation conditions were used. In all cases, most of the biomass was solubilised during the wet oxidation process and only about 20-g solid fraction was obtained from the 60-g added (**Table 37**). This could be due to the fact that the selected wet oxidation conditions were too harsh for the material that had already been processed. Hence, the lignocellulosic structure was probably already partly opened. As the solid fraction took too long time to dry or because the solid fraction 'burned' during the drying process, characterisation of the solid fraction was impossible.

Table 37. *Wet oxidation conditions for crude sugar beet pulp.*

Treat-ment	Pulp (g/L)	Temperature (°C)	Na_2CO_3 (g/L)	Oxygen (bar)	Reaction time (min)	Solid fraction (g)	pH
F1	60	195	6.5	6	15	19.3	-
F2	60	160	6.5	6	10	22.4	-
F3	60	160	6.5	12	10	20.6	6.3
F4	60	170	9	12	10	23.6	7.2

Due to the very high content of pectin in sugar beet pulp (**Table 2**) a relative very high concentration of arabinose was found in the liquid fraction after acid hydrolysis, but otherwise there was not much sugar available for fermentation purposes after wet oxidation (**Table 38**). Due to the low amount of sugar solubilised

and the low weight of the solid fraction, the recovery for the polysaccharides was expected to be low at these conditions.

Table 38. *Characterisation of the liquid fraction obtained from wet oxidation of crude sugar beet pulp.*

Treat-ment	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total sugar (g/L)	2-Furfural (mg/L)	5-HMF (mg/L)
F3	0.65	2.05	2.67	5.37	0	0
F4	0.46	2.09	2.65	5.19	1.7	0.6

So far wet oxidation has not shown to be useful for treatment of sugar beet pulp due to the high amount of pectin present (**Table 2**). The reason for this might be the high content of pectin, which is cross-bounded by present calcium ions in the sugar beet pulp. Risø have plans to redress this problem by using wet oxidation at lower pH-values thus without addition of Na_2CO_3. The low pH will presumably result in an ion exchange of the calcium (Ca^{++}) with hydrogen ions (H^+). In this way calcium could be washed out of the material and the pectin will become available for the process. Risø will try several different approaches in the near future.

3.7 Summary

3.7.1 Wheat straw: Effect of harvest year

The chemical composition for the different harvest years (1990, 1993, 1994 and 1997) only showed minor variations (**Table 1**) (except from 1990 (Bjerre and Schmidt, 1997)), however, the straws looked very different. The straw 1990 and 1994 was nice yellowish, whereas the straw 1997 and in particular straw 1993 was dark and greyish. This colour difference might be due to that the straw 1993 and 1997 had been lying on the fields after harvest or that there had been considerable rain in periods. This have then lead to a certain beginning microbial degradation of the straws, which did not show in the chemical composition analysis.

The straw 1994 behaved somewhat different during the wet oxidation process than straw 1993 and 1997 using the same wet oxidation conditions (**Figure 32**), as much more non-cell wall material was solubilised from the solid fraction. On the other hand, much more lignin was solubilised from straw 1997 compared to the two other harvest years. This also resulted in a higher concentration of carboxylic acids in the liquid fraction. The cellulose convertibility (62-66%) was similar for wheat straw from all harvest years (**Figure 33**) at these wet oxidation conditions.

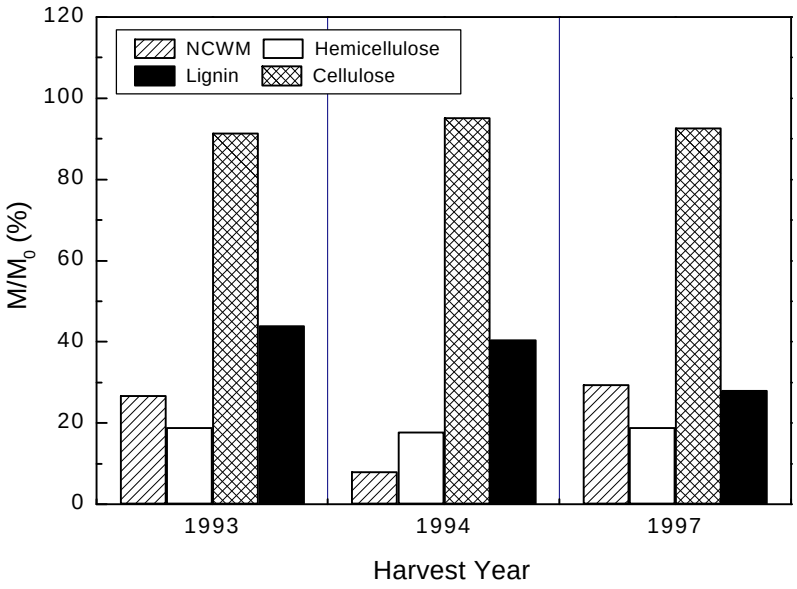

Figure 32. *The relative characteristic (M/M_0) of the solid fraction (non-cell wall material (NCWM), hemicellulose, lignin and cellulose) of wet-oxidised wheat straw from three different harvest years: 1993, 1994 and 1997 (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

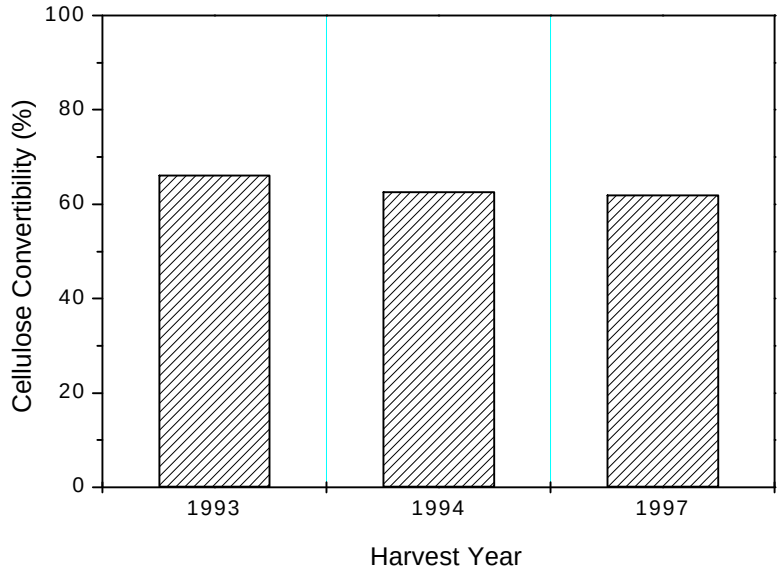

Figure 33. *The cellulose convertibility of the solid fraction for wet-oxidised wheat straw from three different harvest years: 1993, 1994 and 1997 (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

The amount of solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) was different for the different harvest years at the same wet oxidation conditions (**Figure 34**). Wheat straw 1993 gave the highest amount of hemicellulose, however, the conditions used were also found by optimising wet oxidation of straw 1993 (Schmidt and Thomsen, 1998). Wheat straw 1997 performed also well at these

conditions giving only slightly less solubilised hemicellulose, indicating that straws from these two years were similar. Wheat straw 1994 did not performed well at these conditions giving only about 60% of the amount found for the two other harvest years.

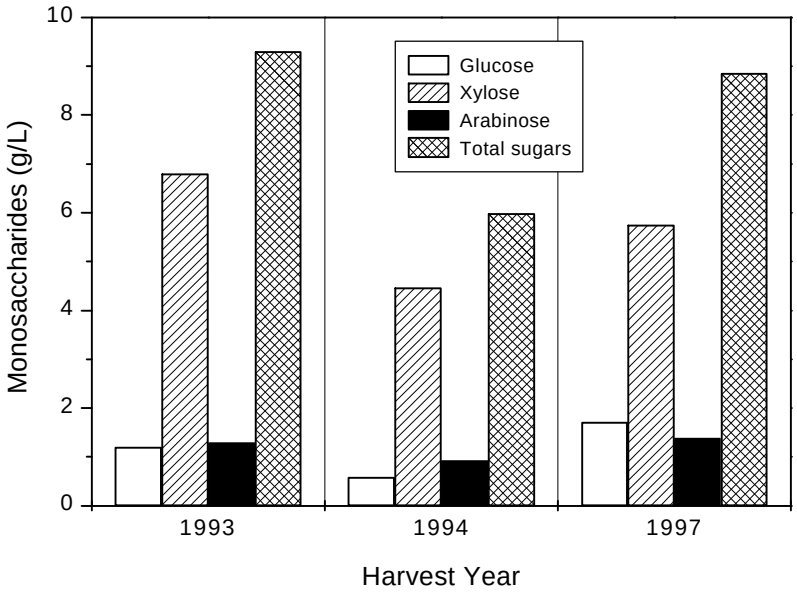

Figure 34. *The hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) of wet-oxidised wheat straw from three different harvest years: 1993, 1994 and 1997 (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

The recovery of hemicellulose and cellulose was quite different from wet oxidation of wheat straw from the three harvest years (using the same conditions) (**Table 39**). Straw 1993 gave the lowest recovery for cellulose whereas the two other years resulted in full recovery of the cellulose. On the other hand, straw 1993 also gave the highest recovery for hemicellulose, slightly higher than straw 1997, but significantly higher than straw 1994, whereas less than 50% of the hemicellulose could be recovered. The overall recovery was highest for wheat straw 1997.

Table 39. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) from the different harvest years. (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes).*

Harvest year	1993	1994	1997
Hemicellulose	58.0	48.3	56.5
Cellulose	95.8	99.6	99.7
Total polysaccharides	76.0	77.4	81.1

3.7.2 Comparing wheat straw with birchwood and willow

As described above the wet oxidation temperature had an effect on solubilisation of hemicellulose from wheat straw 1993, birchwood and willow (measured as monosaccharides after acid hydrolysis) (**Figure 35**). The effect on the solubilisation of hemicellulose from wheat straw was significant, where a temperature of 185°C gave the highest concentration of xylose and arabinose in the liquid fraction but no effect of temperature could be observed on the glucose concentration (Schmidt and

Thomsen, 1998). At 185°C nearly 3 times more hemicellulose was solubilised (about 10 g/L) than at 150°C. During wet oxidation, the temperature also had a considerable effect on the solubilisation of hemicellulose from willow. Again a temperature of 185°C seemed to give the highest concentration of solubilised hemicellulose. At 185°C about twice the amount of hemicellulose was solubilised (about 5.5 g/L) than at 170°C. The temperature also affected the solubilisation of hemicellulose from birchwood, but not in the same extend as for wheat straw and willow. A temperature of around 190°C gave the highest concentration of solubilised hemicellulose (about 4.5 g/L) in the liquid fraction. For willow and birchwood, the concentration of solubilised hemicellulose was still quite low compared to that of wheat straw, hence, very little carbohydrate was available directly for fermentation (Schmidt and Bjerre, 1997).

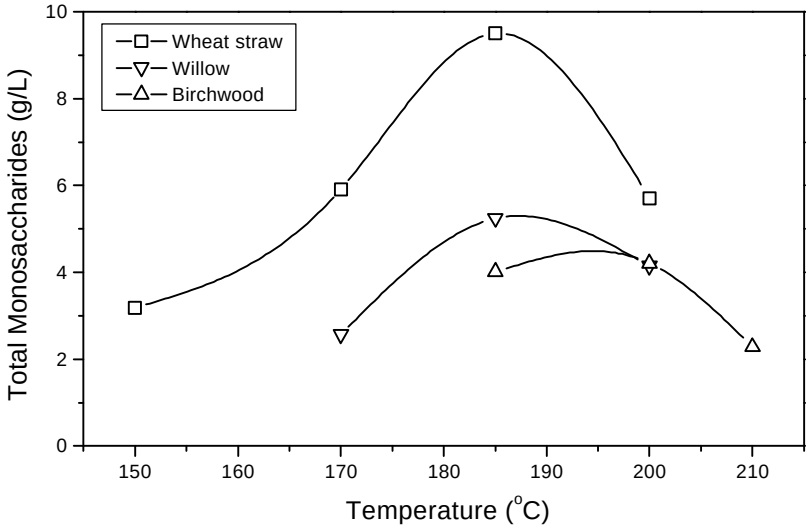

Figure 35. *Solubilisation of hemicellulose (measured as total monosaccharides after acid hydrolysis) from wheat straw 1993,willow and birchwood as a function of the wet oxidation temperature (60 g/L biomass, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes). (Schmidt and Bjerre, 1997).*

Previously, a much higher yield of xylose had been achieved from steaming of birchwood compared to alkaline wet oxidation (Korte *et al*., 1991; Schmidt *et al*., 1996). This could be addressed to the addition of sodium carbonate, which decreased the acidic autohydrolysis during the process (**Table 40**). Autohydrolysis occurs when acetyl groups of hemicellulose are hydrolysed at elevated temperatures in the presence of water to give acetic acid, lowering the pH to 3-4, which permits hydrolysis of the hemicelluloses (Biermann *et al*., 1984). Wet oxidation (without alkaline) gave a slightly higher concentration of solubilised hemicellulose for wheat straw and willow compared to pre-treatment without oxygen. For both materials, wet oxidation gave significantly higher amount of solubilised hemicellulose in the absence of alkaline than in the presence of alkaline. However, much lower amount of hemicellulose was solubilised from willow than from wheat straw.

For wheat straw and willow, absence of both oxygen and carbonate in the treatment, resulted in the lowest concentration of solubilised hemicellulose (**Table 40**), whereas for birchwood these conditions remarkably gave the highest amount of solubilised hemicellulose. The major effect of alkaline was observed for birchwood, where more than half the amount of solubilised hemicellulose was obtained due to an interruption of the autohydrolysis. The difference between the different materials

was probably due to the different distribution of the hemicellulose, lignin and cellulose components in the materials (**Table 1**), amount of acetyl-groups and the type of linkages between them.

Table 40. *The solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) from pre-treatment of wheat straw 1993, willow, and birchwood (Alkaline wet oxidation: 60 g/L biomass, 6.5 g/L Na_2CO_3, 12 bar O_2, 185°C (straw, willow)/200°C (birchwood), 15 minutes). (Schmidt and Bjerre, 1997).*

Pre-treatment	Wheat Straw 1993		Willow		Birchwood	
	Total Sugar (g/L)	pH	Total Sugar (g/L)	pH	Total Sugar (g/L)	pH
Alkaline wet oxidation	9.3	5.9	5.2	4.9	4.2	4.6
Wet oxidation	11.2	3.0	8.2	3.0	8.0	2.4
No oxygen	8.7	7.0	4.4	7.0	2.2	7.5
No oxygen/alkaline	7.8	4.9	2.7	3.7	9.3	3.5

For all three materials, the addition of oxygen and/or alkaline to the pre-treatment process had a large effect on formation of furfurals (2-furfural and 5-hydroxymethyl-2-furfural (HMF)) (**Table 41**) (Schmidt and Bjerre, 1997; Schmidt and Thomsen, 1999). The 2-furfural was formed by degradation of pentoses and HMF by degradation of glucose. By addition of alkaline to the process (high pH) only low concentrations of 2-furfural and HMF were formed for the 3 materials. Whereas high concentrations of the furfurals were formed in the absence of alkaline. Treatment of birchwood gave higher concentrations of 2-furfural than treatment of wheat straw and willow. Whereas treatment of willow formed high concentration of HMF compared to birchwood. No HMF was formed from wheat straw at the applied process conditions, even though a higher glucose concentration was found (Schmidt *et al*., 1996) than for willow and birchwood. Further optimisation of the wet oxidation of willow and birchwood will be carried out in the future.

Table 41. *Formation of 2-furfural and 5-hydroxymethyl-2-furfural (HMF) from hydrothermal treatment of wheat straw 1993, willow, and birchwood (Alkaline wet oxidation: 60 g/L biomass, 6.5 g/L Na_2CO_3, 12 bar O_2, 185°C (straw, willow)/200°C (birchwood), 15 minutes). (Schmidt and Bjerre, 1997).*

Pre-treatment	2-Furfural/Pentoses (mg/g)			HMF/Glucose (mg/g)		
	Wheat Straw	Willow	Birch	Wheat Straw	Willow	Birch
Alkaline wet oxidation	<1	1	7	0	10	23
Wet oxidation	7.8	30	64	0	456	11
No oxygen	<1	0	0	0	0	0
No oxygen/alkaline	8.2	37	45	0	283	105

4 Steaming Pre-treatment

Steaming pre-treatment was performed in order to compare the findings with wet oxidation with a more widely applied pre-treatment process and to evaluate the relative efficiency of the wet oxidation process. This process was carried out at Institute of Wood Science, Hamburg, Germany, in Dr. Jürgen Puls's laboratory. The difference between steaming and steam explosion is ascribed to the way the pressure is released after the process. In steaming, the pressure is released slowly, whereas in steam explosion the pressure is released instantaneously. However, most results indicate that there are no real differences in the process efficiencies for fractionation of biomasses (J. Puls, personal communication).

4.1 Wheat Straw 1993

During our visit to the Institute for Wood Science in Hamburg, steaming experiments with the wheat straw 1993 as used in the wet oxidation experiments were performed. Three different temperatures was applied, 180°C, 190°C and 205°C, at a reaction time of 10 minutes.

4.1.1 Characteristics of solid fraction

The composition of the solid fraction did not vary much with the steaming temperature (**Figure 36**). Only the content of hemicellulose decreased slightly with temperature. An increasing temperature led to a slight increase of the cellulose convertibility up to 35%, however, this was much less than obtained by using wet oxidation (Schmidt and Thomsen, 1998). One reason could be the lack of a catalyst in the steaming process.

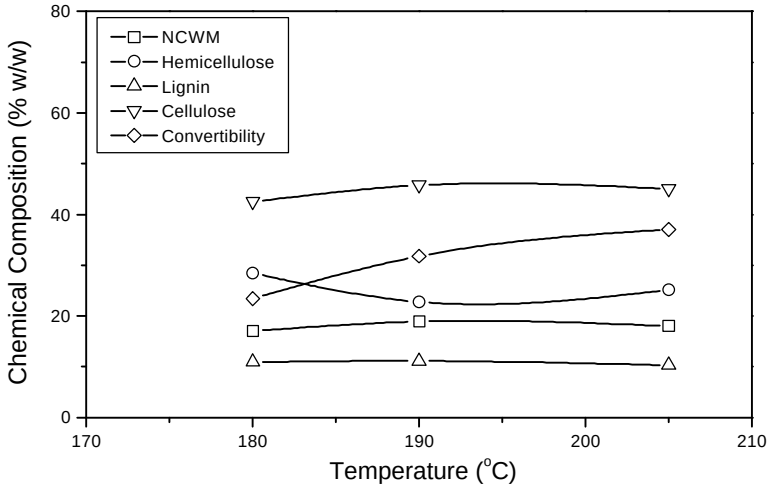

Figure 36. *Effect of steaming temperature on the chemical composition (non-cell wall material (NCWM), hemicellulose, lignin and cellulose) and cellulose convertibility of wheat straw 1993 (200 g straw, 10 minutes).*

The fractionation of wheat straw 1993 by steaming was less efficient compared to wet oxidation as only little lignin was removed (20%) from the solid fraction (**Figure 37**). The low lignin removal resulted in a low convertibility (**Figure 36**). Up to 60% of the hemicellulose had been removed by the steaming process, which was

significantly lower than for wet oxidation that typically removed 80% of the hemicellulose. The steaming process also removed (or decomposed) about 20% of the original cellulose in the solid fraction, whereas during wet oxidation the cellulose fraction was not solubilised by the treatment.

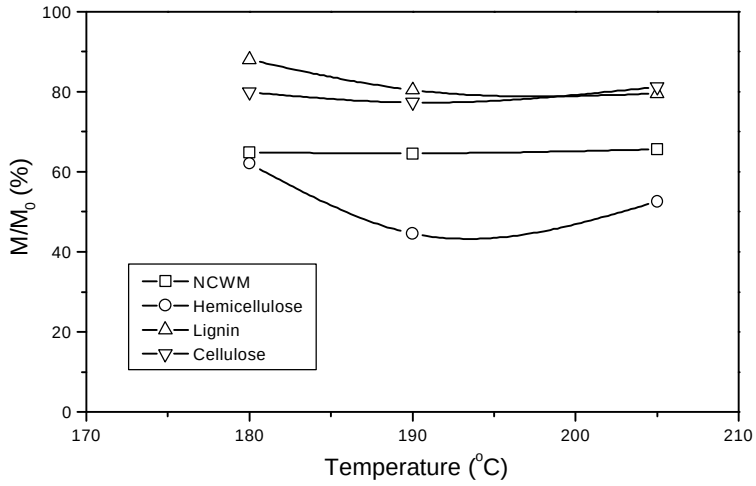

Figure 37. *Effect of steaming temperature on the remaining non-cell wall material (NCWM), hemicellulose, lignin and cellulose in the solid fraction (M/M_0) of wheat straw 1993 (200 g straw, 10 minutes). M: Mass of component in solid fraction. M_0: Mass of component in starting material.*

4.1.2 Characteristics of liquid fraction

The amount of solubilised hemicellulose available for fermentation purposes (**Table 42**) was, as expected, lower for steaming than for wet oxidation due to the low removal of hemicellulose from the solid fraction (**Figure 37**). The sugar composition was analysed at the Institute of Wood Science in Hamburg, Germany. The concentration of solubilised hemicellulose was calculated per 200 g straw, per 100 g straw and per 60 g straw in order to compare the values directly with wet oxidation (which is given per 60 g straw). The highest concentration was obtained at 190°C with a total sugar concentration of 4.5 g/L (based on 60 g straw). However, the value was still only about half of what was obtained for alkaline wet oxidation (Schmidt and Thomsen, 1998). Xylose is the main sugar component, the glucose, arabinose and galactose and only very low amount of rhamnose and mannose.

Table 42. *Effect of steaming temperature on the hemicellulose solubilisation (g sugar) (measured as monosaccharides after acid hydrolysis) from wheat straw 1993 (200 g straw, 10 minutes).*

Temperature	g sugar/200 g straw			g sugar/100 g straw			g sugar/60 g straw		
(°C)	180	190	205	180	190	205	180	190	205
Rhamnose	0.12	0.21	0.19	0.06	0.10	0.09	0.04	0.06	0.06
Mannose	0.22	0.33	0.31	0.11	0.17	0.16	0.07	0.10	0.09
Arabinose	1.16	2.01	1.55	0.58	1.00	0.77	0.35	0.60	0.46
Galactose	0.63	1.02	0.85	0.31	0.51	0.42	0.19	0.30	0.25
Xylose	3.12	9.28	8.37	1.56	4.64	4.18	0.94	2.78	2.51
Glucose	1.53	2.32	2.16	0.76	1.16	1.08	0.46	0.69	0.65
Total	6.79	15.15	13.43	3.39	7.58	6.71	2.04	4.55	4.03

Sugar composition analyses were carried out by Dr. J. Puls, Hamburg, Germany.

The formation of carboxylic acids increased with temperature (**Figure 38**) as observed for wet oxidation (Schmidt and Thomsen, 1998). However, the amount of formed carboxylic acid was much lower for steaming than for wet oxidation, due to the less efficient fractionation for steaming and in particular due to the lack of oxygen which converted reactive components to low molecular weight compounds (**Figure 2**) (Taylor and Weygandt, 1974).

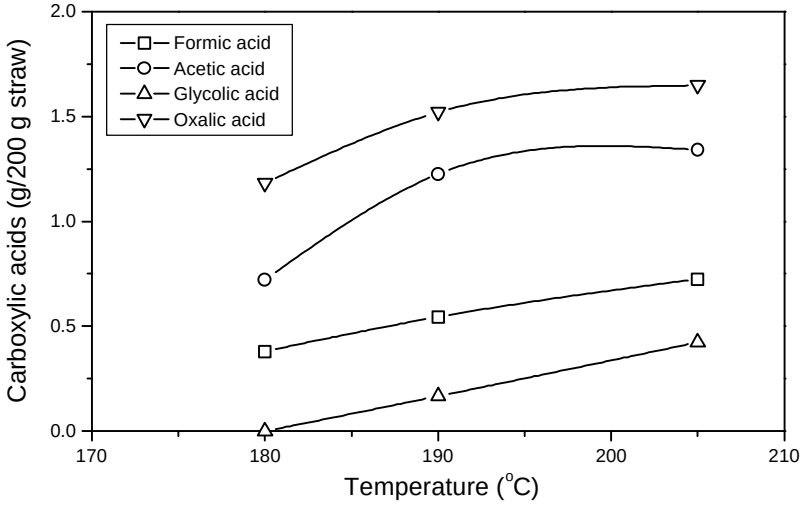

Figure 38. *Effect of steaming temperature on the formation of carboxylic acids from wheat straw 1993 (200 g straw, 10 minutes).*

The formation of 2-furfural from degradation of the pentoses in the hemicellulose increased with increasing steaming temperature (**Table 43**). The amount of formed 2-furfural was significantly higher than for alkaline wet oxidation (Schmidt and Thomsen, 1999) in addition to the lower amount of solubilised hemicellulose (**Table 42**). In all steaming experiments, 2-furfural was formed, although mostly at the higher temperatures. The solubilisation of hemicellulose was very low in the steaming experiments compared to alkaline wet oxidation, hence, the ratio of furfural/pentoses was a factor of 5 to 26 higher (**Table 44**). The higher 2-furfural formation during steaming (compared to alkaline wet oxidation) way be addressed to the lower pH-value during the treatment as seen for wet oxidation (without alkaline) and hydrothermal treatment (**Tables 10** and **44**). For steaming, the formation of 2-furfural was not only due to the effect of pH but also due to the effect of temperature. The steaming experiments were not analysed for formed 5-hydroxymethyl-2-furfural (degradation of glucose).

Table 43. *Effect of steaming temperature on the formation of 2-furfurals from wheat straw 1993 (200 g straw, 10 minutes).*

Temperature (°C)	180	190	205
pH	5.7	4.8	4.9
2-furfural (mg/200 g straw)	7.1	24.2	87.1
2-furfural (mg/100 g straw)	3.6	12.1	43.6
2-furfural (mg/60 g straw)	2.1	7.3	26.1

Table 44. *The comparison of 2-furfural formation (per 100 g straw) from wheat straw 1993 by alkaline wet oxidation (60 g/L straw, 185°C, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes) and steaming at 3 different temperatures (200 g straw, 10 minutes).*

Pre-treatment	pH	Pentoses[b] (g/100 g)	2-Furfural (mg/100 g)	2-Furfural/Pentoses Ratio (mg/g)
Alkaline wet oxidation	5.9	13.5	5.0	0.3
Wet oxidation	3.0	15.9	127	7.8
Alkaline hydrothermal	7.0	13.1	3.0	0.2
Hydrothermal	4.9	11.2	92	8.2
Steaming (180°C)[a]	5.7	2.14	3.6	1.7
Steaming (190°C)[a]	4.8	5.64	12.1	2.2
Steaming (205°C)[a]	4.9	4.96	43.6	8.8

a: Carried out by Dr. J. Puls, Hamburg, Germany.
b: Xylose and arabinose.

4.1.3 Recovery of polysaccharides

The steaming temperature did not affect the recovery of the two polysaccharides, hemicellulose and cellulose, significantly (**Table 45**). However, the recovery for cellulose was considerably lower for steaming than for wet oxidation, whereas the recovery of hemicellulose was slightly higher (Schmidt and Thomsen, 1998). This higher recovery for hemicellulose was explained by the fact that more hemicellulose remained in the solid fraction (**Figure 37**) compared to that of wet oxidation (Schmidt and Thomsen, 1998). In conclusion steaming without a catalyst was not very efficient for fractionation of wheat straw.

Table 45. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) after steaming of wheat straw 1993 (200 g straw, 10 minutes).*

Temperature (°C)	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
180	71.1	81.7	76.8
190	61.8	80.0	71.6
205	65.7	83.8	75.4

4.2 Comparing Wheat Straw with Birchwood

4.2.1 Recovery of xylose

The effect of the alkaline wet oxidation and steaming temperature on the hemicellulose solubilisation and recovery from wheat straw 1993 and birchwood was investigated. The previously determined optimum alkaline wet oxidation conditions for wheat straw (Schmidt and Thomsen, 1998) was also applied for birchwood. Additionally, optimum steaming conditions for birchwood (Korte *et al*., 1991) was applied for steaming of wheat straw. The recovery of xylose was calculated by assuming that 85% of the hemicellulose in the starting material was xylose. The calculation was carried out as shown below for alkaline wet oxidation of 60 g wheat straw at 150°C.

$$Recovery\ of\ xylose\ (\%) = \frac{xylose\ produced\ (liquid)}{xylose\ in\ (untreated\ straw)} \cdot 100\%$$

$$= \frac{xylose\ concentration\ (g/L) \cdot volume\ (L)}{hemicellulose\ concentration\ (g/g) \cdot mass\ (g) \cdot 0.85} \cdot 100\%$$

$$= \frac{1.882\ g/L \cdot 1\ L}{0.328\ g/g \cdot 60\ g \cdot 0.85} \cdot 100\% \quad = \quad 11.4\%$$

The optimum steaming conditions for treatment of birchwood was not optimal for the treatment of wheat straw and *vice versa* (**Figure 39**). Steaming looked more promising for pre-treatment of birchwood whereas the alkaline wet oxidation process looked more efficient for pre-treatment of wheat straw. For birchwood, a higher yield of xylose (and total sugars) was achieved in filtrates from steaming compared to the alkaline wet oxidation process. An optimum temperature was obtained at about 200°C for both processes. For wheat straw, the alkaline wet oxidation treatment was more efficient than steaming with an optimum temperature of 185°C compared to 200°C for steaming. A similar recovery of xylose was achieved for steaming of birchwood as for alkaline wet oxidation of wheat straw. However, the temperature range for high recovery was wide for steaming of birchwood but very narrow for wet oxidation of wheat straw.

Steaming of wheat straw gave extremely low recovery of xylose in the liquid fraction (about 15%) (**Figure 39**). This was mainly due to the low fractionation of the wheat straw by this process and hence most hemicellulose (and xylose) remained insoluble. Steaming is an autohydrolysis which occurs when acetyl groups of hemicellulose are hydrolysed at elevated temperatures in the presence of water to give acetic acid, lowering the pH to 3-4, which permits hydrolysis of the hemicelluloses (Biermann *et al*., 1984). The amount of acetyl groups in wheat straw is significantly lower than in birchwood (Puls, personal communication). This low acetyl content might explain the inefficient autohydrolysis of wheat straw by steaming.

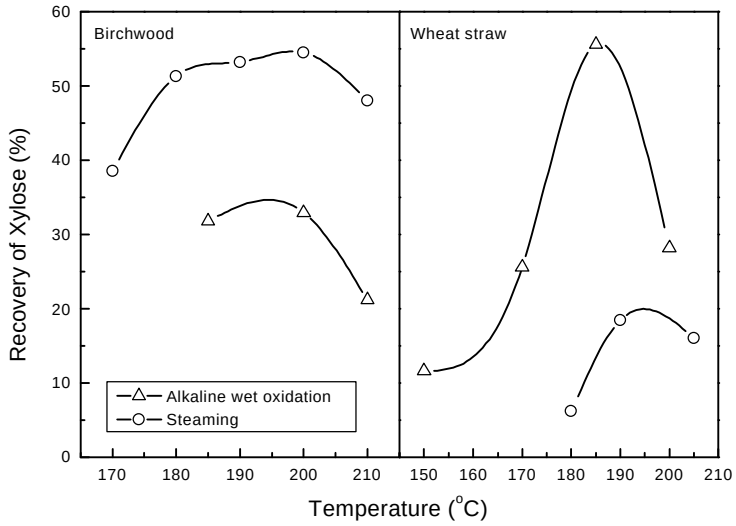

Figure 39. *The recovery of the xylose from wheat straw from 1993 and birchwood pre-treated by alkaline wet oxidation (60 g/L biomass, 6.5 g/L Na_2CO_3, 12 bar O_2, 15 minutes) and steaming (200 g biomass, 10 minutes). Values in % of the xylose in the raw materials. (Schmidt et al., 1996).*

5 Steam Explosion Pre-treatment

Due to the fact that the steam explosion equipment was installed at Risø National Laboratory very late in the project (February 1998) only one type of biomass was investigated using this pre-treatment, namely wheat straw 1997.

5.1 Wheat Straw 1997

As the steam explosion reactor was of a total volume of 5 litres a higher content of straw compared to wet oxidation process could be treated per run. A mass of 200 g was chosen in order to be able to compare the results directly with the steaming experiments carried out at the Institute of Wood Science in Hamburg (see under "Steaming Pre-treatment"). No catalysts (chemicals) were added to the process. Six experiments were carried out investigating the effect of temperature (200 and 190°C) and reaction time (5 and 10 minutes) (**Table 46**), two of the experiments were duplicates (SA1 = SA5; SA2 = SA10). The experiments were also used to establish and demonstrate the equipment. The steam explosion treatment resulted in a loss of dry matter between 23 and 35%, highest at the highest temperature and longest reaction time as expected. After the treatment, the pH-value was around 5 which was fairly high compared to wet oxidation treatment without alkaline addition.

Table 46. *Steam explosion conditions for wheat straw 1997 (200 g straw).*

Exp. no.	Temperature (°C)	Steam pressure (bar)	Reaction time (minutes)	Dry matter (g)	Loss of dry matter (%)	pH
SA1	200	16	10	132	33.8	5.0
SA2	200	16	5	144	27.9	5.1
SA3	190	12.8	10	153	23.8	5.0
SA4	190	12.8	5	154	22.8	4.9
SA5	200	16	10	131	34.7	5.0
SA10	200	16	5	142	28.9	4.8

5.1.1 Characteristics of solid fraction

In all experiments, the solid material had a brownish colour and was characterised by an increase in lignin, cellulose, and ash content and a decreased hemicellulose content in the treated material compared with untreated straw (**Table 47**). Temperature seemed to affect the characteristic of the solid fraction much more than time. High temperature (200°C) resulted in lower hemicellulose and lignin content and higher cellulose content than low temperature (190°C). The content of non-cell wall material was not affected by the steaming explosion conditions. Some variation between the results from the duplicate steam explosion experiments was observed and was probably due to inexperience with running the equipment. These 6 experiments are the first steam explosion experiments carried out at Risø National Laboratory. It should be remembered that these experiments were preliminary. Hence, this variation is expected to diminish as work with the equipment progresses.

The convertibility of cellulose was increased from 14% in the untreated material to 25 to 40 % in steam-exploded materials. The highest convertibility was obtained at the highest temperature, 200°C, and longest reaction time, 10 minutes (**Table 47**). This is much lower than that of wet oxidised wheat straw which had gave a convertibility around 65% as the highest and around 39% as the lowest (**Table 21**). The low convertibility in the steam exploded straw may be due to the relatively

higher lignin content as seen for wet oxidised straw (**Figure 7**) (Schmidt and Thomsen, 1999). The higher content of NCWM may also influence the convertibility in a negatively way.

Table 47. *Effect of steam explosion conditions on the chemical composition (non-cell wall material (NCWM), hemicellulose, cellulose, lignin, and ash) (% dry weight) and the cellulose convertibility of solid fraction of wheat straw 1997 (200 g straw). Conditions as described in* ***Table 46****.*

Exp. no.	NCWM (%w/w)	Hemicellulose (%w/w)	Cellulose (%w/w)	Lignin (%w/w)	Ash (%w/w)	Convertibility (%w/w)
Straw	25.1	27.9	37.0	8.7	1.4	14.1
SA1	26.8	7.6	51.9	11.6	2.1	39.4
SA2	24.8	12.1	48.2	11.6	3.3	32.5
SA3	22.7	20.3	46.4	9.2	1.4	25.3
SA4	23.3	19.7	45.8	9.6	1.6	25.5
SA5	24.9	11.5	48.7	12.3	2.6	43.9
SA10	24.8	15.7	47.3	10.5	1.7	35.3

About 30-35% of the original non-cell wall material (NCWM) was removed during the steam explosion (**Table 48**), but this did not vary with the reaction temperature and time. This amount is only half of the removal of NCWM in the wet oxidised processes (**Table 20**). Also the removal of hemicellulose was relatively lower than for wet oxidised treated straw. The best wet oxidation removed 95% hemicellulose (**Table 20**) compared to 82% in SA1 (**Table 48**). This indicated lower fractionation efficiency in steam explosion than in the wet oxidation process. In all steaming experiments more than 5% of the cellulose has been removed from the solid fraction, which was similar to what was obtained for wet oxidation of wheat straw 1997.

Table 48. *Effect of steam explosion conditions on remaining non-cell wall material (NCWM), hemicellulose, cellulose, and lignin (M/M_0) from the solid fraction of wheat straw 1997 (200 g straw). M: Mass of component in solid fraction. M_0: Mass of component in starting material. Conditions as described in* ***Table 46****.*

Exp. no.	NCWM (%)	Hemicellulose (%)	Cellulose (%)	Total Lignin (%)
SA1	70.8	17.9	92.9	88.5
SA2	71.1	31.1	93.9	96.0
SA3	69.0	55.4	95.5	80.8
SA4	71.6	54.6	95.4	85.0
SA5	64.7	27.0	86.0	92.5
SA10	70.4	40.1	91.0	85.7

5.1.2 Characteristics of liquid fraction

The washing procedure resulted in approximately 1.5-L filtrate, which contained solubilised hemicellulose. There were no monomeric sugars present in solution after steam explosion. After acid hydrolysis the monomeric sugar distribution was found as illustrated in **Table 49**. In these experiments, 200-g straw was used and the sugar concentrations were not directly comparable with those of wet oxidation experiments. Hence, the sugar concentrations per litre were converted to concentrations per 60-g straw (**Table 49**). The steam explosion resulted in less than 50% of the total sugars that was obtained by wet oxidation (**Table 22**) with a different composition. Much less relative arabinose and more glucose was found in the mixture. The results from SA1 and SA5 were very different even though the

steam explosion conditions were the same (**Table 46**). This was put down as inexperience in operating the equipment.

Table 49. *Effect of steam explosion conditions on hemicellulose solubilisation (measured as monosaccharides after acid hydrolysis) from wheat straw 1997 (200 g straw). Conditions as described in* ***Table 46****.*

Exp. no.	Filtrate (L)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total Sugars (g/L)	Total Sugars (g/60g straw)
SA1	1.50	3.88	9.84	0.48	14.19	4.26
SA2	1.50	3.75	8.80	0.71	13.26	4.00
SA3	1.55	3.86	5.76	0.78	10.39	3.11
SA4	1.34	3.96	5.99	0.73	10.68	3.20
SA5	1.44	2.11	5.99	0.25	8.35	2.50
SA10	1.44	3.13	7.54	0.66	11.33	3.40

In experiment SA10, the content of 2-furfural and HMF was measured (**Table 50**). The contents are comparable to those of wet oxidation with low carbonate addition and low oxygen pressure (**Table 25**), where the pH-value was around 4.8 as in SA10. This indicates that the formation of furfurals is due to the heating procedure more than of the specific pre-treatment process. As illustrated for alkaline wet oxidation of wheat straw 1997, the formation of furfurals may be prevented if the pH-value was above 6.0 (**Table 25**). No attempts were made to identify other potential inhibitors in these preliminary experiments.

Table 50. *Formation of 2-furfural and 5-hydroxymethyl-2-furfural (HMF) during steam explosion (200 g wheat straw, experiment SA10). Conditions as described in* ***Table 46****.*

2-Furfural		HMF		2-Furfural/Pentoses	HMF/Glucose
(mg/L)	(mg/60g straw)	(mg/L)	(mg/60g straw)	(mg/g)	(mg/g)
60	18	10	3	5.1	2.2

The recovery of the polysaccharides was calculated by means of the equation on page 17 of this report (Schmidt and Thomsen, 1998). When comparing the most promising steam explosion experiment (SA1 and SA2 (**Table 51**)) with the most promising after wet oxidation (I7 and I8 (**Table 26**)), the hemicellulose recovery was lower for steam explosion than for wet oxidation. Furthermore, more hemicellulose was measured in solution after wet oxidation and thereby available for fermentation. A better recovery was obtained on the cellulose fraction after steam explosion (**Table 51**) compared to wet oxidation (**Table 26**) where 5% was lost in experiment I8. However, more cellulose material was convertible (68%) from wet oxidation compared with only 39 and 33% from steam explosion. Hence, steam explosion was considered less efficient than wet oxidation for production of fermentable polysaccharides from wheat straw. Steam explosion gave similar results as steaming, however, two different materials were used wheat straw 1997 and 1993, respectively. Therefore, the results are not directly comparable. Furthermore, two different reactors had been applied for the two studies.

Table 51. *Calculated recovery based on mass balances for the polysaccharide fractions (hemicellulose and cellulose) at different steam explosion conditions (200 g wheat straw). Conditions as described in* ***Table 46****.*

Exp. no.	Hemicellulose (%)	Cellulose (%)	Total Polysaccharides (%)
SA1	42.3	99.9	75.2
SA2	53.7	100.8	80.5
SA3	71.4	102.8	89.3
SA4	68.8	101.9	87.6
SA5	41.1	89.7	68.8
SA10	58.7	96.5	80.3

6 Characterisation of Products

6.1 Quantification of Solubilised Hemicellulose

6.1.1 Acid hydrolysis and purification

Previously, an analytical method for quantifying the solubilised hemicellulose from wet-oxidised lignocellulosic materials was developed based on acid hydrolysis and quantification of the released monosaccharides by HPLC (Bjerre *et al*., 1996b). To avoid interference from salts and non-sugar substances in the HPLC analysis with subsequent damage to the column, acid hydrolysates were deionised before injection (Kaar *et al*., 1991; Puls, 1993). The sulfate ions were removed by precipitation with barium either by barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$) or barium carbonate ($BaCO_3$). The recovery and reproducibility of the analysis were greatly influenced by the way the precipitation was conducted. Therefore, this was investigated in more details.

Hardly any difference could be observed (**Table 52**) in the sugar recovery using the different levels of barium hydroxide or carbonate, when barium was used in less than the equivalent amount (1.15 g hydroxide or 0.72 g carbonate). The release of carbon dioxide during the precipitation with barium carbonate was very slow (up to several days), hence, the time required per analysis increased significantly using carbonate instead of hydroxide. Comparison of chromatograms clearly showed a more effective purification using barium hydroxide without additional loss of recovery (data not shown). Thus barium hydroxide was chosen to be the best precipitation agent.

Table 52. *Sugar yield (g/L) using barium hydroxide ($Ba(OH)_2 \cdot 8H_2O$) (the control) and barium carbonate ($BaCO_3$) as precipitation agents. Values have not been corrected for recovery.*

Hydroxide (g)	Carbonate (g)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total Sugars (g/L)
0.25	-	1.26	5.38	1.20	7.84
-	0.25	1.28	5.63	1.20	8.11
-	0.50	1.32	5.60	1.22	8.14
-	1.00	1.22	5.63	0.85	7.70

The precipitation step was followed by a purification step, where ion exchange was used to remove interfering substances such as inorganic salts and low molecular carboxyl acids (Bjerre *et al*., 1996b). The Risø standard method involved a small ion exchange column, which consisted of a cation (Amberlite GC-120 (100-200 mesh, H^+)) and an anion (Dowex 1x4 (50-100 mesh, OH^-)) exchanger divided into layers. The fabrication of this very efficient column was laborious, hence, the efficiency of the less laborious mixed-bed ion-exchanger (Dowex MR-3) was investigated. The mixed-bed ion exchanger showed to be as efficient as the small column (**Table 53**). However, the amounts of mixed-bed ion exchanger greatly influenced the recovery. By keeping a low level of mixed-bed ion exchanger, reversible binding of sugars to the resin could be avoided resulting in a consistent recovery. The use of this mix-bed ion exchanger (Dowex MR-3) will be implemented in the near future when sugar recovery has been evaluated.

Table 53. *Purification using a mixed-bed ion exchange (Dowex MR-3) and a control using an ion exchange column (Amberlite GC-120 and Dowex 1x4) (Bjerre et al., 1996b). Values are not corrected for recovery.*

Mixed-bed ion exchange (g)	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total Sugars (g/L)
control	1.43	5.44	1.19	8.06±0.05
0.20	1.36	5.62	1.20	8.18±0.14
0.40	1.34	5.55	1.20	8.09±0.08

6.1.2 Enzymatic hydrolysis

Enzymatic hydrolysis can indicate whether the solubilised hemicellulose was available for fermentation, but could also be used for quantification of solubilised hemicellulose. By using enzymatic hydrolysis for quantification instead of acid hydrolysis, which is presently employed (Bjerre *et al*., 1996b), the laborious, tedious, and time-consuming sample preparation and purification of the acid hydrolysates *prior* to HPLC analysis can be avoided.

The enzymatic hydrolysis was optimised using a concentrated enzyme mixture of 4 commercial enzyme preparations (Econase, Ecopulp, Gamanase, Novozym), supplied by VTT Biotechnology and Food Research, Finland (Schmidt *et al.*, 1998). The hydrolysis was compared with the previously optimised acid hydrolysis on the same wet-oxidised wheat straw hemicellulose using the standard procedure developed at Risø National Laboratory (Bjerre *et al*., 1996b) (**Table 54**). The overall monosaccharide yield from enzymatic hydrolysis was only 82% of that of the acid hydrolysis, but the ratio of the two hydrolyses was different for the different monosaccharides. The glucose yield was similar probably due to excess cellulase activity in the VTT-enzyme mixture (Schmidt *et al*., 1998) relatively compared to the amount of solubilised cellulose (glucose). The low yield of the monosaccharides deriving from the hemicellulose (xylose and arabinose) (only 80%). As the VTT-enzyme mixture originated from a different source than the substrate all the necessary enzymes for complete hydrolysis of the solubilised wheat straw hemicellulose from wet-oxidation pre-treatment might not have been induced and produced, which could lead to missing or very low activities of some essential enzymes.

Table 54. *Comparison of the monosaccharide yield from wet-oxidised wheat straw hemicellulose by using acid hydrolysis (4% w/v sulfuric acid, 120°C, 10 minutes) and enzymatic hydrolysis (VTT-enzyme mixture, volume-ratio = 0.042, pH = 5.0, 45°C, 24 hours). (Schmidt et al., 1998).*

Monosaccharides	Acid hydrolysis (g/L)	Enzymatic hydrolysis (g/L)	Sugar Ratio[a] (%)
Glucose	0.74	0.77	103
Xylose	6.47	5.22	81
Arabinose	1.59	1.26	80
Total sugar	8.81	7.25	82

a: The monosaccharide yield ratio of enzymatic to acid hydrolysis.

If an enzyme mixture was produced on solubilised wheat straw hemicellulose from wet-oxidation pre-treatment, the enzymes necessary for complete hydrolysis are expected to be formed with satisfying activity. Such an enzyme mixture should act much more specifically on the hemicellulose present in the pre-treated wheat straw and give a better hydrolysis than the applied VTT-enzyme mixture.

An investigation into the composition of oligosaccharides after the enzymatic hydrolysis might also reveal which specific enzyme activities are missing or limiting, or whether or not inhibitors are present in the wet-oxidised substrate.

6.2 Identification of Potential Inhibitors

The liquid hemicellulose-rich fraction contains numerous monomeric degradation products hydrolysed from the individual constituents in lignocellulose during wet oxidation. Phenolic monomers from lignin degradation, acetic acid from hydrolysis of acetylated sugars and furan derivatives from sugar degradation are considered to be potential fermentation inhibitors (Clark and Mackie, 1984; Delgenes *et al.*, 1996) (**Figure 2**). The low molecular phenolic degradation products from wheat straw pre-treated by wet oxidation have not previously been characterised. Solid phase extraction (SPE) of phenols from complex matrixes such as wine and waste water has in the last years been established as being a reliable extraction technique with high recoveries (Cheung and Wells, 1997; Prater *et al.*, 1980; Soleas *et al.*, 1997). Gas chromatography mass spectrometry (GC-MS) is an established technique to identify and quantify compounds from complex mixtures in low concentrations. Derivatisation by trimethylsilylation (TMS) of hydroxyl groups makes it possible to screen liquid fractions from pre-treated biomass for a vast number of low molecular weight compounds such as phenols and carboxylic acids (Niemelä and Sjöström, 1986).

By silylating the freeze-dried extract, the less volatile and hydrophilic low molecular weight components such as hydroxy carboxylic acids could be identified, however, this method was not optimal for quantification. A method to identify and quantify phenolics was developed based on solid phase extraction (SPE), freeze drying, derivatisation and GC-MS (**Figure 40**).

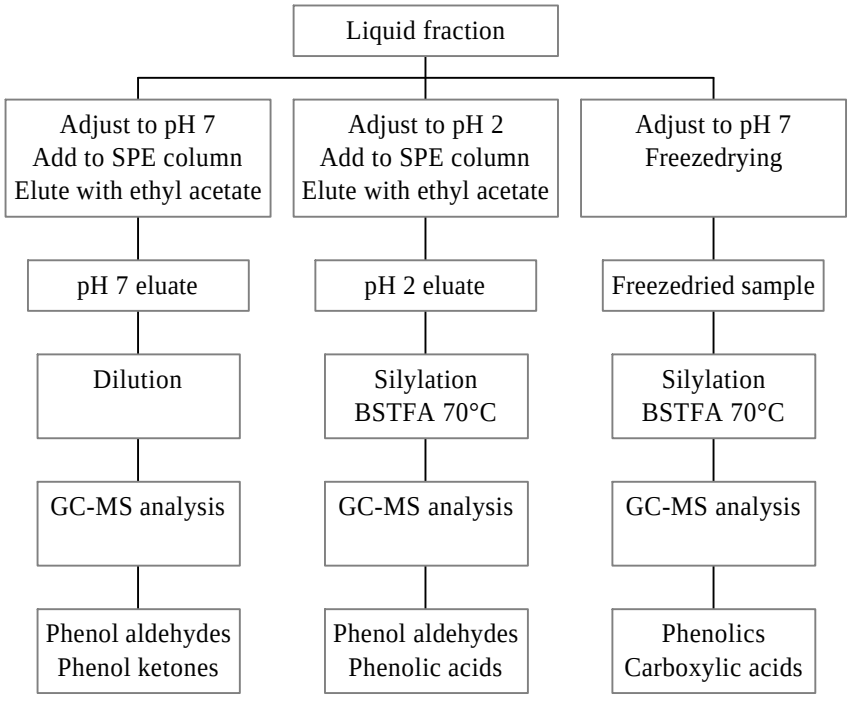

Figure 40. *Sample preparation scheme for determination of phenolics on GC-MS.*

Lignin degradation products can be divided into three main phenolic structures consisting of a para-substituted phenol with zero, one or two methoxy groups ortho to the phenol hydroxyl group. All three basic structures were identified as their phenol aldehydes, ketones/ acetophenones and acids. From the silylated fractions of SPE and freeze-dried extracts, several compounds could be identified by their mass spectra and semi quantified with authentic standards. Due to keto-enol isomerisation of the phenolic ketones and formation of the TMS-ether upon treatment with BSTFA, the SPE extract at pH 7 was analysed without derivatisation. The phenolics were more abundant than the furan derivatives (**Tables 24** and **25**) (Klinke *et al.*, 1999) and a qualitative and semi-quantitative determination of these potential inhibitors was made by GC-MS analysis.

7 Conclusions

- The **alkaline wet oxidation** was an effective process for the fractionation of **wheat straw** into solubilised hemicellulose and a cellulose fraction highly convertible for enzymatic treatment yielding glucose. A high oxygen pressure during the treatment and a low lignin content in the treated fibres resulted in a highly convertible cellulose fraction.

- Different optimal reaction conditions were found from the **different harvest years**. For wheat straw from 1993 and 1997, the optimal reaction conditions were 185°C, 15 minutes reaction time, 12 bars O_2 resulting in 9-10 g/L solubilised hemicellulose (from 60 g straw/L) and 63-67% convertibility of the cellulose fraction. For wheat straw from 1994, the optimal reaction conditions were 195°C, 5 minutes reaction time, 12 bars O_2 resulting in 7.5g/L solubilised sugar and 96% convertibility of the cellulose fraction.

- For **willow,** the optimal pre-treatment was **wet oxidation** (without alkaline) using 185°C, 12 bar O_2, 15 minutes reaction time (treating 60 g willow/L). These conditions gave 8.2 g/L hemicellulose in solution and a 50% convertible cellulose fraction, which was lower than that of wheat straw. Higher recoveries were obtained when treating willow compared to wheat straw. Addition of alkaline significantly decreased fractionation and the degree of enzymatic convertible cellulose.

- For **birchwood**, the best process condition was **hydrothermal treatment** (without oxygen and alkaline addition). At 200°C and 15 minutes reaction time 8 g/L hemicellulose was solubilised from the solid fraction with high recoveries for both hemicellulose and cellulose, however, poor convertibility of the solid cellulose fraction was found (<30%). The addition of oxygen (wet oxidation) also resulted in a high fractionation of the hemicellulose and cellulose fractions but with decreased recoveries of both polysaccharide fractions. Alkaline wet oxidation resulted in the highest convertibility of the cellulose fraction but had a low content of hemicellulose in solution (less than 4 g/L).

- For all tested biomass the formation of **furfurals** was avoided by addition of alkaline addition during wet oxidation. Without alkaline addition the formation of furfurals were higher (up to 130 mg/100 g wheat straw) than for steam explosion (43 mg/100 g straw). The formation of **carboxylic acids** was highest during alkaline wet oxidation condition and highest when treating birchwood (up to 8 g/L). In comparison, steam explosion resulted in a lower formation of carboxylic acids. Minor amounts of **phenolic compounds** were identified after wet oxidation (wheat straw) in mg/L levels.

- In a detailed mass balance on the liquid fraction from alkaline wet oxidation, the solubilised hemicellulose accounted for 33% of the measured total organic carbon (TOC), low molecular weight carboxylic acids for approximately 20%, furfurals for 0.02% and phenolic compound for 1.5%. However, some residual TOC presumably high molecular weight components remained unidentified.

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Further Development of Chemical and Biological Processes for Production of Bioethanol: Optimisation of Pre-treatment Processes and Characterisation of Products

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Abstract (max. 2000 characters)

The efficiency of several processes for pre-treatment of lignocellulose has been investigated to provide feedstock for enzymatic hydrolysis and fermentation. Wet oxidation (WO) (with and without alkaline) has been investigated for wheat straw, birchwood, and willow treating 60 g/L. Three different harvest years of wheat straw were included to evaluate the effect of crop variation from year to year. Comparative studies were made using steaming and steam explosion of wheat straw. Alkaline WO fractionated wheat straw efficiently into solubilised hemicellulose and a highly convertible cellulose fraction. High oxygen (12 bar) during WO and low lignin in treated fibres resulted in highly convertible cellulose. Different optimal reaction conditions were found for different harvest years. For straw 1993 and 1997, conditions were 185°C, 15 minutes resulting in 9-10 g/L solubilised hemicellulose and 63-67% cellulose convertibility. For straw 1994, conditions were 195°C, 5 minutes resulting in 7.5 g/L solubilised hemicellulose and 96% cellulose convertibility. For willow, the optimal pre-treatment was WO without alkaline using 185°C, 15 minutes, giving 8.2 g/L hemicellulose in solution and 50% cellulose convertibility. For birchwood, the best process conditions were hydrothermal treatment (without oxygen and alkaline). At 200°C and 15 minutes, 8 g/L hemicellulose was solubilised with high recoveries for both polysaccharides, however, poor cellulose convertibility was found (<30%). Alkaline WO resulted in the highest cellulose convertibility but low contents of solubilised hemicellulose (< 4 g/L). In general, formation of furfurals was avoided by adding alkaline during wet oxidation. In the absence of alkaline, furfural formation was higher (up to 130 mg/100 g wheat straw) than that of steam explosion (43 mg/100 g straw). Formation of carboxylic acids was highest during alkaline wet oxidation and highest for birchwood (up to 8 g/L). Minor amounts of phenolic compounds were identified after alkaline wet oxidation of wheat straw. Some residual (TOC) presumably high molecular weight components remained unidentified.

Descriptors

WHEAT, STRAW, WILLOWS, BIRCHES, WOOD, HEMICELLULOSE, CELLULOSE, LIGNIN, WET OXIDATION PROCESSES, ALKALINE HYDOLYSIS, STEAM, AUTOHYDROLYSIS, COMPARATIVE EVALUATIONS, FURFURAL, ACID HYDROLYSIS, ENZYMATIC HYDROLYSIS

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